ASPECTS OF THE BIOLOGY AND CULTIVATION OF THE WARATAH (*TELOPEA SPECIOSISSIMA* R. BR).

BY

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CERTIFICATE OF ORIGINALITY

The text of this thesis contains no material which has been accepted as part of the requirements for any other degree or diploma in any university or any material previously published or written unless due reference to this material is made.

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ABSTRACT

Using intermittent mist, cuttings of waratah *(Telopea speciosissima)* were propagated with a high success rate (nearly 100 per cent) when shoots were taken at the commencement of growth in spring. Cuttings were treated with benomyl (250 ppm) to control *Guignardia citricarpa* (Black Spot) and indolebutyric acid (IBA - 2000 ppm) was applied by the quick dip method. Selected clonal material propagated using this method was more vigorous and had a greater survival rate than seedlings when transplanted into the field.

Waratah seedlings grown in perlite or a yellow earth gave an increased shoot weight in response to added P fertilizers, i.e. up to 100ppm available P in the yellow earth and up to 6.4 m moles P/100 g perlite in the perlite medium. Waratah seedlings responded to added N fertilizer when applied to the perlite medium but addition of N to the yellow earth caused a high mortality rate (94%). Results suggested that death of plants may have been due to an interaction between the N fertilizer and the susceptibility of plants to *Phytophthora cinnamomi* rather than to a N toxicity. There was an inhibitory effect on shoot growth when K fertilizer was added to the yellow earth and only a small response to added K in the perlite medium.

The growth of proteoid roots was generally inhibited by N fertilizers, especially NH_4^+ which was more readily taken up than NO_3^-. High P levels (greater that 3.2m moles P/100g perlite) also inhibited the formation of proteoid roots. Inoculation with *P. cinnamomi* reduced both shoot and non-proteoid root dry weight only at high levels of added N. Proteoid root growth was only reduced by inoculation with *P. cinnamomi* at low (zero) N levels.

The vase life of untreated *T. speciosissima* inflorescences harvested

at their optimum stage with respect to vase life (0 to 5 per cent of styles reflexed) was 13 days at 20°C. Vase life was not limited by stem blockage, supply of respirable substrates or by ethylene. HQC (8-hydroxyquinoline sulphate, 0.075 to 0.600 mM) inhibited stem blockage. Storage at 0.5°C for 9-10 days reduced the subsequent vase life of inflorescences by 25-30 per cent. Iprodine inhibited the development of *Botrytis cinerea* on inflorescences stored in plastic bags at 20°C.

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CHAPTER 1.

INTRODUCTION

Telopea speciosissima R. Br. (common name waratah) is increasing in importance as a cut flower in Australia. Plantings of over 0.5 ha, which have been inspected by the author, are listed in Table 1.1. By far the largest planting is 34 ha at Gembrook, Victoria, which was planted in 1982. Recently, commercial production of waratahs as cut flowers has also begun in Israel, New Zealand and South Africa. *T. speciosissima* has many features which make it suitable as a cut flower i.e. a long vase life, a stem length of at least 30 cm, bright red colour and a potentially high productivity (Parvin *et al.*,1973). A typical plant is shown in Plate 1.1. Commercial growers and home gardeners often experience difficulties in the growing of waratahs but some growers claim that it is a more profitable crop than vegetables such as tomatoes (Plate 1.2). The most serious problem facing growers is the mortality of plants when transplanted into the field (Plate 1.3). It is not uncommon for plantations to be replanted two or three times before an adequate stand is established. The reason for these high losses has been attributed to many causes including the fertility of the soil, water stress and disease. There is little published information on the effects of these factors on waratah survival and growth and there are no soundly-based recommendations for the commercial production of *T. speciosissima*. However, once established, waratah plants may persist in plantations for more than 40 years (Table 1.1).

The vigour of plants established from seed varies considerably in most commercial plantings. While exceptional individuals may produce up to 150 blooms per year, many plants produce only 2 or 3. There is an obvious opportunity for the selection of high-yielding clones, but commercial growers

Table 1.1 Plantings of *T. speciosissima* over 0.5 ha in area inspected by the author.

Site	Age at 21st June, 1984	Area (ha)	Soil type
Central Mangrove via Gosford, NSW	2.5 yrs	1.0	Yellow earth
Gembrook Dandenong Mts, VIC	2.5 yrs	34.0	Krasnozem
Kariong via Gosford, NSW	40 yrs +	2.0	Yellow earth
Kurrajong Hts Blue Mts, NSW	3 yrs	0.6	Yellow earth
Mangrove Mt via Gosford, NSW	7 yrs	1.5	Yellow earth
Monbulk Dandenong Mts, VIC	30 yrs	1.0	Krasnozem
Peats Ridge via Gosford, NSW	8 yrs	0.8	Yellow earth

Plate 1.1 Garden specimen of *T. speciosissima (1.5 m high)* Note numerous large terminal flowers. The plant is about 5 years old. Photographed at Kuringai Wildflower Garden 20th September, 1977.

Plate 1.2 *Telopea speciosissima* planting at Mangrove Mountain, Central Coast region, N.S.W. Once established under suitable conditions *Telopea speciosissima* grows vigorously. Note the bare patch in foreground due to losses within one month of transplanting.

(a)

(b)

Plate 1.3 *Telopea speciosissima* plantings at Somersby (a) and Kariong (b) Central Coast region, N.S.W. showing poor establishment of transplants. Note how losses tend to be localised.

who have attempted vegetative propagation usually achieve a very low percentage of rooted cuttings. There is little published information on vegetative propagation of *T. speciosissima*.

The aim of this thesis is to investigate aspects of the biology and cultivation of the waratah which remain an impediment to its commercial exploitation as a cut flower.

CHAPTER 2 REVIEW OF LITERATURE

Botanical Description

Telopea speciosissima R.Br. (common name waratah) is a member of the family *Proteaceae*, subfamily *Grevilleoidea*, tribe *Embothrieae*, subtribe *Embothriinae* (Wills, 1959; Beadle *et al.* 1976; Johnson and Briggs, 1975). The other members of the genus *Telopea* are *T. oreades*, F. Muell (Gippsland waratah), *T. mongaensis* (Monga waratah) and *T. truncata* (Tasmanian waratah). The flowers of these three are smaller than those of *T. speciosissima*. The subtribe *Embothriinae* contains two other genera which share a common ancestor with *Telopea* (Johnson and Briggs, 1975). They are *Embothrium*, a genus of 1 species of evergreen tree native to southern Chile and Argentina in South America, and *Oreocallis*, a genus of 4 species of evergreen trees and shrubs, native to South America, Australia and New Guinea.

Telopea speciosissima is described by Wills (1959) and Beadle *et al.* (1976) as a woody, erect shrub growing to 4 m in height with alternate leaves 10-25 cm long, 2-4 cm wide, usually dentate and sometimes divided and with prominent venation on the upper surface. The general habit of the plant is shown in Plates 1.1 - 1.3. The flowers are red but vary considerably in shade. Flowers are pedicellate and are arranged in a dense ovoid to globular inflorescence 8-15 cm in diameter. The structure of the flowers and the inflorescences are shown in Plate 2.1. The flowers at the base of the inflorescence open first. The fruit is a recurved coriaceous follicle (7.5 - 9.0 cm) with two rows of ovules. The seeds are winged, flat and are usually freely produced.

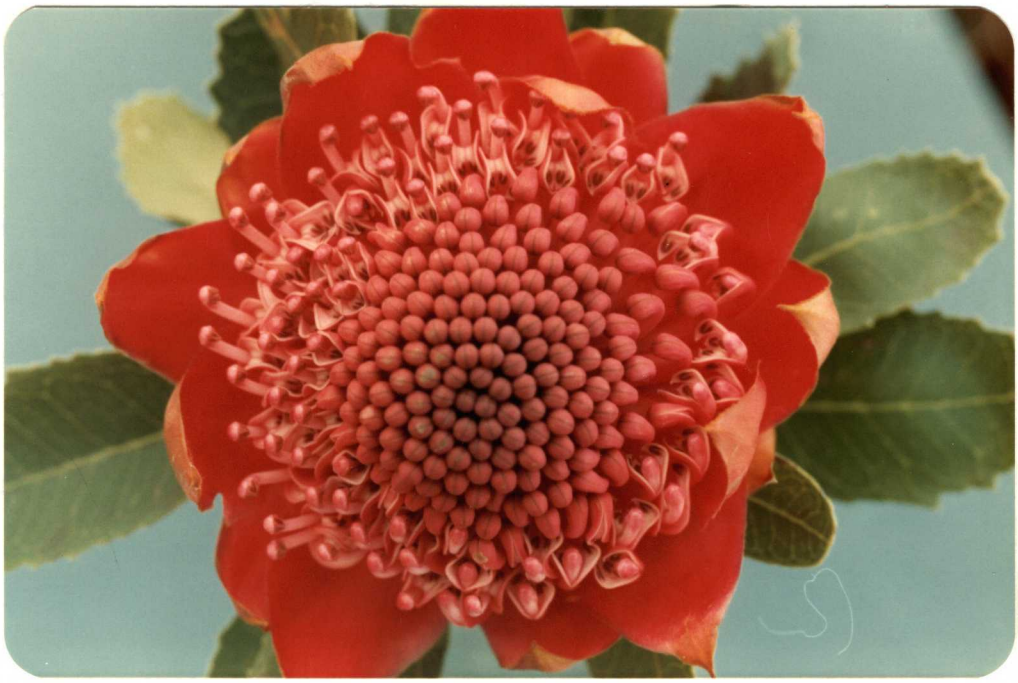


Plate 2.1 (a) Inflorescence of *T. speciosissima* (x 0.6)

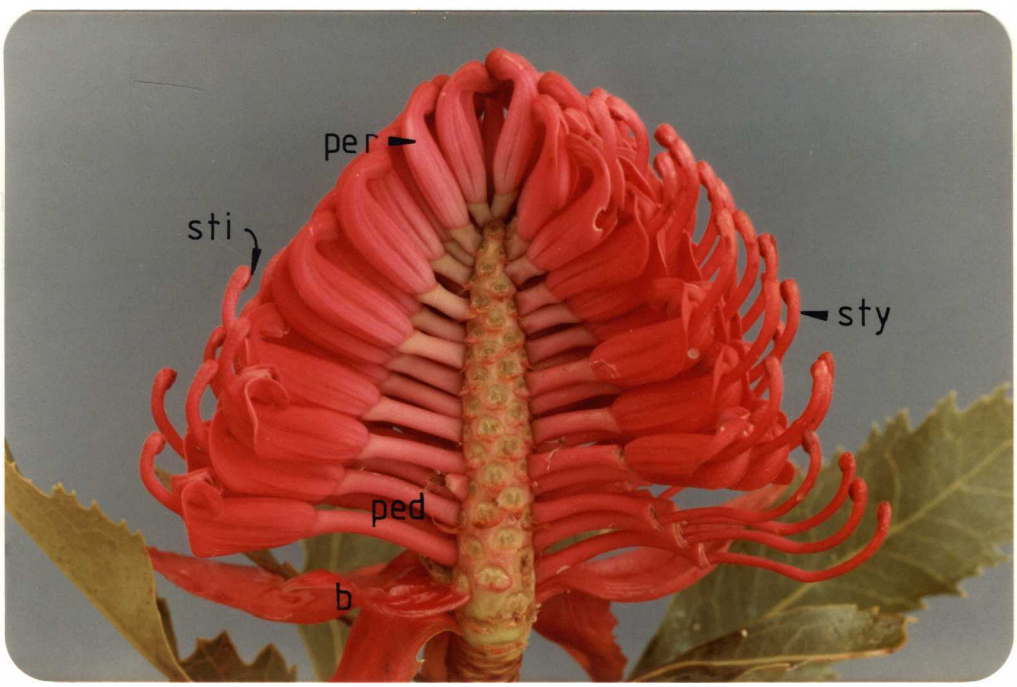


Plate 2.1 (b) Inflorescence of *T. speciosissima* with flowers and bracts removed on one side (b - bracts, ped - pedicel, per-perianth tube, sti-stigma, sty-style (x 0.6)

Plants of the genus *Telopea* possess proteoid roots, a specialised common feature of the family *Proteaceae* (Plate 2.2). These specialised roots are an adaption to infertile soils (Purnell, 1960). They were defined by Purnell (1960) as being dense clusters of rootlets of limited growth borne along lateral roots. Only one non-proteaceous species, *Viminaria juncea*, a legume, possesses similar structures (Lamont, 1972a).

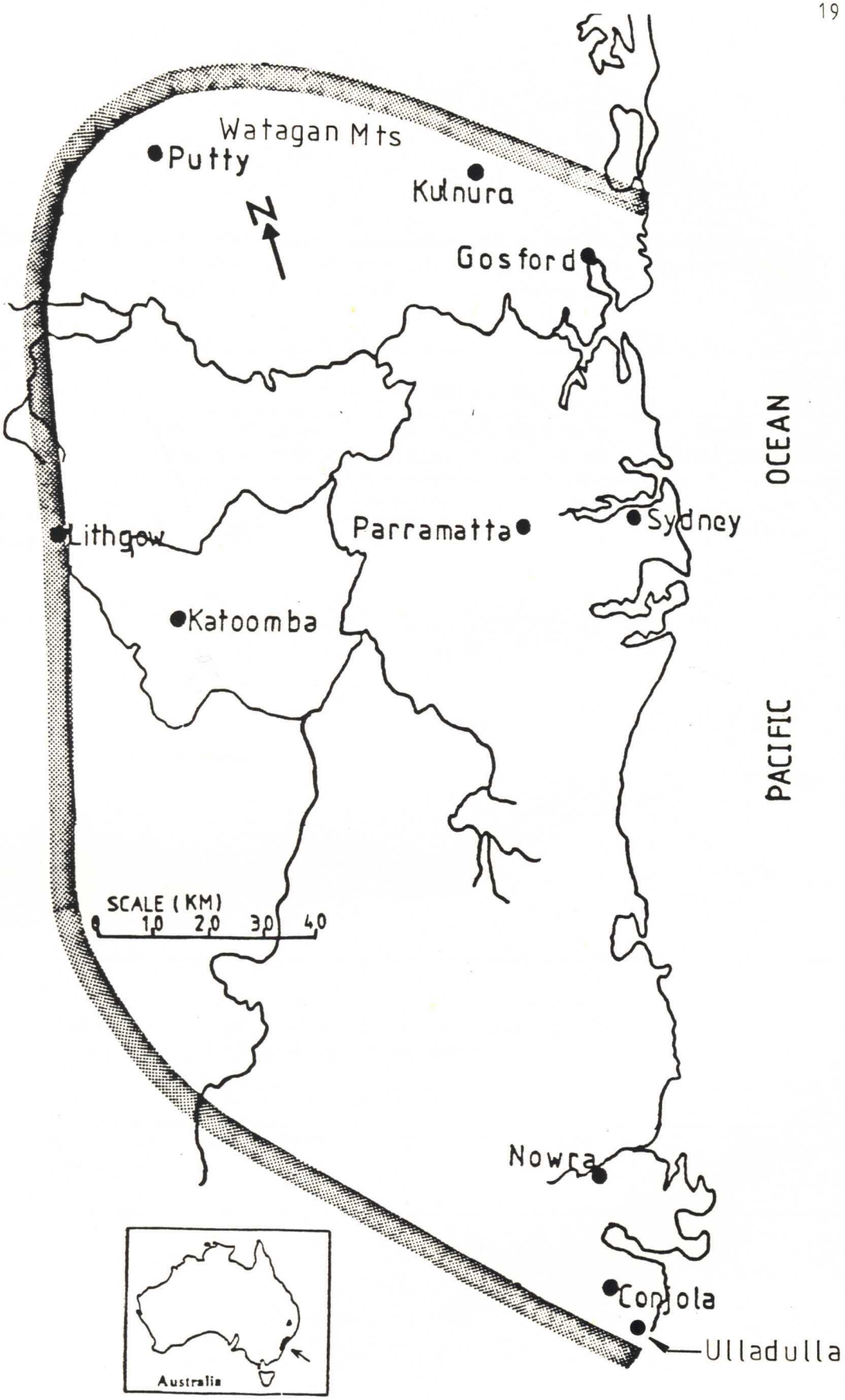
Telopea speciosissima seedlings possess a lignotuber, a structure which acts as a regenerative organ after the aerial portions of the plant have been destroyed by fire or drought (Stone, 1966). When the mature plants are pruned multiple stems are usually produced from the lignotuber and this leads to greater flower production.

Distribution

Except for one isolated occurrence at Glen Elgin in the ranges north-east of Glen Innes (NSW) *T. speciosissima* occurs only in a strip of coastal New South Wales 200 km long and 100 km wide which is bounded in the north by the Gosford-Putty region; in the south by Conjola; in the west by the western slopes of the Blue Mountains and in the east by the Pacific Ocean (Wills, 1959) (Figure 2.1). Within this area *T. speciosissima* is restricted to soils derived from Triassic sandstones, mainly of the Hawkesbury and Narrabeen sandstone groups (Hannon 1958; Wills, 1959; Wright *et al.*, 1975; Beadle *et al.*, 1976; Driver and Hawkins, 1978; Specht, 1978).

Temperature and rainfall vary widely within the area of occurrence of *T. speciosissima* and the climatic data at three locations is given in Table 2.1a. Although the rainfall is fairly evenly distrubuted throughout the year, on an average monthly basis, there is a slight peak in the late

Plate 2.2 Proteoid roots of *T. speciosissima* seedlings suspended in water (1 division = 0.5 mm)



- limit of distribution except for occurrence at Glen Elgin (Gibraltar Range, N.S.W.).

Figure 2.1. Natural distribution of *Telopea speciosissima*.

Table 2.1a Average annual rainfall and average temperatures at three locations in New South Wales where waratah occurs in the wild.

Climatic parameter	Location		
	Mount Wilson (Elevation 1066 m)	Mangrove Mountain (Elevation 274 m)	Somersby (Elevation 150 m)
Average annual rainfall (mm)	1283	897	1315
Average temperature (°C)			
Summer			
- maximum	23	24	26
- minimum	12	14	16
Winter			
- maximum	10	16	18
- minimum	3	6	5

Table 2.1b Average monthly rainfall at Somersby - 60 year average (mm)

Month											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
138	143	148	134	112	132	86	76	68	93	91	94

summer-early autumn period. Precipitation is least in spring (Table 2.1b). There is also a tendency for long periods without rain and these dry periods may exceed one month. The rainfall also tends to be of high intensity. For example 14mm falls per wet day at Somersby (New South Wales Department of Agriculture data). The soils on which *T. speciosissima* occurs in the wild are yellow earths. The yellow colour is due to the high iron content of the parent sandstone. These soils are classified as Gn 2.74 in the Northcote classification (Wright *et al.*, 1975) and they have been heavily leached. The organic fraction is usually concentrated in the top 5 cm of the profile. The texture of the soil is a sandy loam and it is uniform to a depth of about one metre. The vegetation type in the area of the natural distribution of *T. speciosissima* tends to be dry sclerophyll forest (Beadle *et al.*, 1976).

Telopea speciosissima has evolved in soils of low natural fertility in which most of the soil nutrients are in the surface organic layer. The high levels of iron and aluminium oxides, about 25% of the clay fraction, (Wright *et al.*, 1975), together with a high content of amorphous-alumino-silicates (Bradley and Vimpany, 1969) lead to significant fixation of P from P fertilizers (Wright *et al.*, 1975). The climate (heavy rain-falls and long dry periods) may also reduce available P (Simpson and William, 1970).

Telopea speciosissima has evolved some special adaptions to this harsh environment, especially the development of proteoid roots. However, many *Proteaceae* which have evolved under similar conditions of low natural fertility will grow satisfactorily in a wide range of soil types and soil fertility levels (Parvin *et al.* 1973). The same may apply to *T. speciosissima*.

Proteoid Roots

(a) Anatomy

Proteoid roots are formed along the axes of lateral roots. In *Telopea* neither the axis of the proteoid root nor the rootlets are branched. The whole proteoid root is 0.62 - 3.75 cm long in *Telopea oreades* F. Muell (Purnell, 1960). Proteoid roots have a root structure similar to lateral roots but they do not undergo secondary growth. Proteoid roots become non-functional in 2 - 3 months from initiation (Purnell, 1960). Most proteoid roots are formed on new lateral roots and, as the root elongates, a succession of proteoid roots may be formed. These provide an intense exploration of the soil due to the large surface area of the root system

(b) Initiation of proteoid roots

Proteoid roots do not form under sterile conditions (Lamont, 1974; Lamont and McComb, 1974; Malajczuk and Bowen, 1974). No fungi or bacteria have been observed within young healthy proteoid roots and neither have any external associations such as ectomycorrhizas been seen. It is thought that initiation of proteoid roots is caused by rhizosphere micro-organisms (Purnell, 1960; Lamont and McComb, 1974). Lamont (1974) provided indirect evidence that the bacterial rather than fungal micro-flora of the soil is responsible for proteoid root initiation by showing that bactericides inhibit their formation.

Micro-organisms may be responsible for the initiation of proteoid roots, but subsequent growth is controlled by nutrient availability (Lamont, 1974).

Lamont (1972b) described four phases in the relationship between

proteoid root formation and increasing nutrient availability. These phases are as follows:-

(a) an increase in proteoid root production (weight) as non-proteoid root growth increases;

(b) a decrease in proteoid root production as non-proteoid root growth increases;

(c) a decrease in proteoid root production as non-proteoid root growth decreases;

(d) an absence of proteoid roots as non-proteoid root growth decreases.

He claims that this relationship occurred for both increasing levels of N and P as well as for increasing levels of organic matter in the soil.

Absolute deficiencies of nutrients may also inhibit production of proteoid roots. Proteoid roots were formed on *Grevillea robusta* when watered with a complete nutrient solution. Deletion of N, Fe, Mg, K, Ca or trace elements inhibited the development of proteoid roots. Proteoid roots did form however, in the absence of added P and S (Moore and Keraitis, 1971).

(c) The role of proteoid roots

Proteoid roots are not essential to the survival of the plant (Groves, 1964). However, proteoid roots in the field constitute 40% or more of the root system (Malajczuk and Bowen, 1974; Lamont, 1975) and they are situated in the humus-rich layer where most soil nutrients are found. Accordingly proteoid roots are likely to be of major importance in the mineral nutrition of the plant (Lamont, 1975). Proteoid roots are very efficient in the uptake of phosphate from the soil and may also enhance

the uptake of other nutrients in a manner similar to mycorrhizal associations (Jeffery, 1967; Malajczuk and Bowen, 1974). Increased ability to take up nutrients has ecological importance because the plants of the family *Proteaceae* generally grow in soils with a low nutrient status (Parvin *et al.*, 1973).

Cultivation and Mineral Nutrition

Telopea speciosissima grows naturally in soils which are deficient in N, P, K, Ca, Mg, Mo, Zn and Cu and which are too infertile for the normal growth of exotic pasture species and fruit trees (Wright *et al.*, 1975). Typical soils supporting populations of *T. speciosissima* contain about 60 ppm K. This is the lower critical level of K for many exotic pasture species. The available P is also very low (3 ppm) and it is noteworthy that 30 ppm available P is the low critical limit for exotic pasture species (Wright *et al.*, 1975). Typical total soil N levels are 330 ppm but little of this is available to the plant because it is an integral part of the soil organic matter. Only about 25 ppm of the N pool is thought to be available each year (Hannon, 1958). Most of the nutrients available in the soil type are derived from the decomposing litter layer at the surface (Hannon, 1958).

Telopea speciosissima has proved to be difficult to grow in cultivation (Brackpool, 1964; Anon, 1972; Ben-Jaacov *et al.*, 1978) but this may not be directly related to soil type, because, in many instances *T. speciosissima* grows well on a wide range of soil types including sand, heavy clays and slightly alkaline soils. The main requirement for the successful cultivation of *T. speciosissima* is that the soil be well drained (Brackpool, 1964; Stone, 1966; Anon, 1972; Parvin *et al.*, 1973; Ben-Jaacov *et al.*, 1978; Wrigley and Fagg, 1979). *T. speciosissima*

appears to have no special requirements for growth as compared with other members of the family *Proteaceae*. It will grow satisfactorily in commercial plantations of *Proteaceae* when given the same fertilizer regime as the other plants (Parvin *et al.*, 1973; Ben-Jaacov *et al.*, 1978). However, as stated by Parvin *et al.*, (1973), recommendations for fertilizer programmes vary from none to quite high levels of inorganic fertilizers. In contrast, most Australian writers recommend low levels of organic fertilizers for the cultivation of *T. speciosissima* (Stone, 1966; Searle, 1971; Anon, 1972; Anon, 1973).

The injudicious use of fertilizers may be partly responsible for the unreliability of *T. speciosissima* in cultivation. Specht (1963) showed that for many Australian heath plants growing under soil fertility conditions similar to those of *T. speciosissima*, application of superphosphate (54 kg/P/ha) or sodium nitrate (35 kg/N/ha) did not affect germination but adversely affected the establishment of plants. No reason for this was advanced by Specht but low levels of phosphate are toxic to some Australian *Proteaceae* (Grundon, 1972; Jones *et al.*, 1978; Nichols *et al.*, 1979; Nichols and Beardsel, 1981; Nichols *et al.*, 1981; Thomas, 1981, 1982).

Goodwin (1982) stated that *T. speciosissima* is sensitive to phosphate. He concluded that 360 g P per cubic metre (but not 120 g P/m^3) added as superphosphate was toxic to *T. speciosissima*. There is some doubt as to the actual rates used by Goodwin. In his paper there is disagreement between the levels of P claimed and those calculated from the rates of fertilizer given in the text and their NPK ratio.

The level and source of N is also important for the growth and survival of some plants (Hewitt, 1966). Proteas, for example, utilize

NH_4^+ much more efficiently than NO_3^- (Claassens and Folisher, 1982) but various species of *Eucalyptus* prefer NH_4^+ to NO_3^- (Moore and Keraitis, 1971).

Phytophthora cinnamomi Rands is pathogenic to *T. speciosissima* and can be a serious problem in cultivation. Infection with *P. cinnamomi* results in "sudden death" of the plants (Bertus Pers. comm.). Although *P. cinnamomi* is widespread throughout the natural habitat of *T. speciosissima* (Pratt *et al.*, 1971; Broadbent and Baker, 1974) it does not appear to be a major cause of plant death in natural populations. *Pythium* and *Rhizoctonia* are also known to attack seedlings of *Telopea* and other *Proteaceae* (Frazer, 1964; Parvin *et al.*, 1973).

No reports of the specific nutrient requirements of *T. speciosissima* have been published. There is a need to determine the effects of commonly used fertilizers on the growth and survival of *T. speciosissima* especially in relation to susceptibility to infection by *P. cinnamomi* and other "water moulds".

Propagation

(a) From seed

Telopea seed germinates readily (Bagshaw, 1962; Scott, 1964; Anon, 1966; Anon, 1973) when it is fresh (Anon, 1973). Seedlings are prone to damping-off (Bagshaw, 1962; Anon, 1966; Gray, 1969; Anon, 1972) by *Pythium, Phytophthora* and *Rhizoctonia* (Fraser, 1964; Parvin *et al.*, 1973). *Botrytis* spp. may also infect the plants (Parvin *et al.*, 1973). Control of these pathogens can be achieved with fungicides, by avoiding over-watering and by use of free-draining potting mixes (Anon, 1972).

(b) From cuttings

Vegetative propagation has a number of advantages over seed propagation, but there is no published experimental work on the vegetative multiplication of *T. speciosissima*. Rousseau (1967) states that vegetative propagation of South African *Proteaceae* for cut flowers is important, because of the variability of seedlings,to maintain true-to-type characters for colour, stem length of blooms, flowering time and disease resistance as well as for multiplication of hybrids. There is evidence that *T. speciosissima* propagated from seed are very variable in flower size and shape, leaf shape, plant size (Wills, 1959; Beadle *et al.*, 1976) and there is some evidence of variation in disease resistance (Brackpool, 1964). It would be highly desirable if superior clones of *T. speciosissima* were available for both commercial production and the home garden.

The use of growth regulators, fungicides, time of year and maintenance of turgidity of the cuttings whilst roots are formed are important factors in the propagation of woody plants from cuttings.

(b) (i) Growth regulators

Auxins play a major regulatory role in the growth and development of plants, e.g. control of cell elongation, tropisms, cambial division and vascular differentiation, apical dominance, abscission and formation of adventitious roots on stem cuttings (Gauthert, 1969; Wareing, 1973; Hartman and Kester, 1975; Goldsmith, 1977). Auxins are widely used in commercial plant propagation to promote adventitious root formation. IBA (indolebutyric acid) is the most common auxin used commercially because it is non-toxic over a wide range of concentrations and it is effective in promoting adventitious roots on cuttings from large numbers

of plant species (Hartman and Kester, 1975). Rousseau (1967) compared the effectiveness of IBA, IAA (indoleacetic acid) and NAA (naphthalene - acetic acid) in promoting adventitious root formation of some *Proteaceae*, both singly and in mixtures. Generally IBA alone or in combination with IAA gave the best results. Many specialist plant propagators in the United States use IBA in vegetative propagation of Proteas (Parvin *et al.*, 1973). Auxins are applied commercially to cuttings as powder preparations, by soaking in dilute solutions or by dipping in a concentrated solution (Hartman and Kester, 1975).

Powder Preparation

The auxin may be either mixed with talc or first dissolved in ethanol then mixed with talc and dried. Heung and MacGuire (1973) claimed that the latter method promotes better rooting of cuttings. Although solutions of IBA, IAA and NAA are often more effective in promoting root formation than the corresponding powder preparations (Heung and MacGuire, 1973) this is not so for some plants (Stoutmezer, 1938, 1954). Rousseau (1967) recommended using a powder carrier for 7 out of 21 species of *Proteaceae* which were examined; the others responded best to dipping in a concentrated solution. Powder preparations are readily available but uniform results are difficult to obtain due to the variable amount of material that adheres to the cutting. This is influenced by a number of factors including texture of the stem and the amount of moisture at its base (Hartman and Kester, 1975).

Dilute Soaking Method

The bases of cuttings are soaked in a dilute solution of auxin for about 24 hours before planting. The method is little used today because results

are more variable than with the concentrated-dip method due to differences in uptake associated with changes in temperature and evapotranspiration during the soaking period (Hartman and Kester, 1975).

Concentrated-solution dip method

The concentrated solution is prepared by dissolving the chemical in ethanol then diluting to 50% with water. The base of the cutting is then dipped into the solution for a short time, usually 5-10 seconds (Heung and MacGuire, 1973; Hartman and Kester, 1975).

The majority of the *Protaeceae* examined by Rousseau (1967) had more rapid root formation when ethanol rather than talc was used as a carrier. Ellyard (1976) examined the effect of talc and liquid carriers for auxins on a number of Australian natives including *Grevillea laurifolia* and found the concentrated solution dip method to be superior in all cases. Maximum uptake for IAA in *Ilex* cuttings is three times faster with this method than with talc. However, it is possible some plants respond better to a slow rate of auxin uptake (Heung and MacGuire, 1973).

(b) (ii) Use of Fungicides

Some systemic fungicides, especially benomyl, can improve the rooting of woody cuttings (Fiorino *et al.*, 1969; MacGuire and Vallone, 1971; Thielges and Hoitink, 1972). This effect has been attributed to both disease control (Thielges and Hoitink, 1972) and to the effect of benomyl's cytokinin-like properties (Skene, 1972). It is one of a number of fungicides to possess both these properties although the other fungicides (e.g. captan and thiobenzole) possess less cytokinin-like activity

(Skene, 1972). Benomyl appears to act as an adenine analogue (MacGuire and Flock, 1975). The effect of benomyl on adventitious root formation is not consistent and may vary between cultivars and/or seasons. This may be due to changes in the endogenous cytokinin-auxin ratio during the growing season (Wareing, 1973; MacGuire and Flock, 1975).

Application of captan may also increase the survival and quality of rooted cuttings when used as a powder dip following IBA treatment or when mixed with the IBA in talc powder (van Doesburg, 1962; Wells, 1963). Pre-soaking of cuttings in benomyl will improve survival in a number of species (Fiorino *et al.*,1969). A mixture of benomyl (5%) and captan (25%) in talc gave maximum rooting in *Pinus strobus* while added IBA had no effect (Thielges and Hoitink, 1972). Treatment of rhododendron cuttings with benomyl and thiobendazole controlled the fungal pathogen, *Cylindrocladium,* and greatly improved rooting (Hoitink and Schmittenner, 1970). Thus, the use of fungicides, especially benomyl which has both fungicidal and cytokinin-like activity, is worth investigating in regard to the vegetative propagtion of *T. speciosissima.*

(b) (iii) Physiological State of the Cuttings

Time of year and its influence on the physiological condition of the cutting material (e.g. growing or dormant) is an important factor in determining the ease of adventitious root formation in a wide range of plants (Lamphear and Meahl, 1961; Hartman and Loretti, 1965; Wareing, 1973; MacGuire and Flock, 1975; Avidan and Lavee, 1978; Gil-Albert and Biox, 1978; Schmidt, 1978). Rousseau (1967), in a study of adventitious root formation in the *Proteaceae,* recommended different collection times for different species and these collection times varied from mid-winter to mid-summer. The response to applied auxin may also vary between seasons

(Lamphear and Meahl, 1961; Howard, 1965, 1968; MacGuire and Flock, 1975). Cytokinin levels in both poplar (Hewitt and Wareing, 1973) and begonias (Heide, 1965) varies with the photoperiod. MacGuire and Flock (1975) have postulated that the changes in the cytokinin-auxin ratio, rather than the auxin level alone may be responsible in part for the different responses of plants to exogenously applied growth regulators. They also speculated that different plants may not respond to the same degree to the same cytokinin-like material.

Growing conditions may also affect the ability of plants to form adventitious roots by altering the level of carbohydrate reserves in the stem. It has been recommended that lignified shoots of *T. speciosissima* from the current season's growth after the completion of flowering should be taken. Root formation on this material generally takes 4-6 weeks (Anon, 1972). However, no supporting information for this recommendation is given and no mention is made of use of applied growth regulators. Thus, there is a need to examine the effect of time of year and other environmental conditions on the rooting of *T. speciosissima* cuttings.

(b) (iv) Use of Intermittent Mist for Vegetative Propagation

The use of intermittent mist in propagation beds has been successfully applied to the propagation of many *Proteaceae* (Rousseau, 1967; Parvin *et al.*, 1973) including *Grevillea* and *Persoonia* (Lamont, 1975; Ellyard, 1976). Other species may be difficult to propagate. When *Telopea monga-ensis* is placed under mist most of the leaves abscind and a thick callus is formed, but no roots (Anon., 1973). *Macadamia* is another member of the Australian *Proteaceae* that is difficult to propagate from cutting (Hartman and Kester,

1975; Tukey, 1978).

Misting prevents dessication of soft succulent (unlignified) material and keeps slow rooting, older, material alive until roots can form. Under the high light intensities and high temperature conditions encountered in the Sydney region misting is a successful means of keeping cuttings fully turgid (Slezinski and Davidson, 1973; Loach and Walley, 1978; Tukey, 1978). The mist propagation procedure permits significant photosynthesis and allows the maintenance of high levels of carbohydrates during the striking of cuttings (Tukey, 1978). It may also reduce the level of abscisic acid (ABA) in the cutting due to the minimisation of water stress (Lee and Tukey, 1972; Hemphill and Tukey, 1973). Mist propagation can also induce the formation of root promoting substances (Wott and Tukey, 1967; Lee and Tukey, 1971). Soaking cuttings in water can also stimulate root development (Kawase,1964, 1971). This was attributed by Kawase (1964) to an increase in ethylene production but there is conflicting evidence as to the role of ethylene in root initiation. (Kirshnamoorthy, 1970; Mullins, 1972).

Mist also reduces the temperature of the leaf, decreases the respiration rate and increases the amount of carbohydrate available for root formation (Hartman and Kester, 1975; Loach and Whalley, 1978; Tukey, 1978).

Post Harvest Physiology

Telopea speciosissima inflorescences are produced in sufficient numbers and have a long enough vase life to justify their cultivation for cut flower production (Parvin *et al.*, 1973; Lamont, 1982). No information

is available on either the normal vase life of inflorescences under standardised conditions or on factors affecting vase life. The factors which are usually important in determining the vase life of other cut flowers are:- maintenance of water balance, availability of respirable substrate,ethylene, temperature and other environmental conditions, water quality and effect of damaging micro-organisms (Halevy and Mayak, 1980, 1981).

(a) Maintenance of water balance

The maintenance of a high level of turgidity in cut flowers is necessary for the development of immature flowers or buds to maturity and the maintenance of normal metabolic activity (Rogers, 1973). Turgidity depends on the balance between the rate of water loss or utilization and the water supply (Marousky, 1968a; Rogers, 1973). Wilting leads to a reduction in flower quality and vase life (Nelson, 1978). Water stress in *Protea* causes a blackening of leaves (Jacobs, 1982) and this is probably due to cell membrane dysfunction (Whitehead, 1979).

Stem-plugging, the blocking of the xylem vessels, is an important cause of reduced water uptake in cut flowers (Mastalerz, 1977; Nelson, 1978) and the main mechanisms by which stem-plugging occurs are micro-biological and physiological.

A large number of chemicals have anti-microbial activity and may extend the vase life of flowers by inhibiting microbial growth in the xylem contents. This in turn prevents the blockage of the water-conducting elements. Chemicals which have been tested commercially or experimentally and have been found to be effective in inhibiting stem

blockage include 8-hydroxyquinoline sulphate (HQS), 8-hydroxyquinoline citrate (HQC), copper sulphate, silver nitrate, zinc acetate, aluminium nitrate, amphyl (Rogers, 1973) and benzalkonium chloride (Casp *et al.*, 1980). Generally HQS and HQC have the widest spectrum of effectiveness and greatest margin of safety (Scholes and Boodley, 1964; Larsen and Scholes, 1965; Nelson, 1978). The salts of 8-hydroxyquinoline may also reduce physiological stem-plugging (Zentmeyer, 1943; Nelson, 1978) by inhibiting enzymes in the stems (Pokorny, 1955; Aarts, 1957; Marousky, 1971).

Both sucrose and salts of 8-hydroxyquinoline can affect stomatal opening and thus water loss from the cut flower. Partial stomatal closure, for up to 2 days, has been observed in roses (Marousky, 1969), chrysanthemums (Stoddard and Miller, 1962) and gladioli (Marousky, 1968a, 1968b) held in HQC solutions.

Preservatives containing a mixture of sucrose and HQC have been shown to extend the post harvest life of a range of *Protea* cut flowers, principally by the prevention of leaf blackening. Preservative solutions are widely used in the commercial production of cut proteaceous flowers in Hawaii, especially *Protea* and *Leucadendron* (Paull *et al.*, 1980).

However, these solutions are not used extensively by commercial growers in South Africa who are faced with similar post harvest problems as the growers in Hawaii (Jacobs, 1982). This may be due to the much lower "field gate" price received by the South Africans and post harvest treatments are thus likely to be uneconomic (Lamont, 1982). There is no record in the literature of the use of preservatives to extend the post harvest

life of *Telopea speciosissima* inflorescences.

(b) Supply of respirable substrates

An important influence on the potential keeping life of cut flowers is the reserve of respirable substrates within the flower. These are principally carbohydrates (Hannon ,1964; Rogers, 1973; Mastalerz, 1977). Utilization of respirable substrates continues in the flower after it has been harvested, but there is generally little or no photosynthesis by cut flowers because light levels encountered in typical post harvest environments are low. In the absence of photosynthesis there is a depletion of reserves and senescence is accelerated (Mastalerz 1977; Nelson, 1978). Where light is of sufficient intensity to allow photosynthesis during holding and use there is often an imporvement in both the quality of the foliage and the keeping quality of the flowers due to increased carbohydrate levels (Woltz, 1966; Woltz and Waters, 1967; Nelson, 1978). Sucrose is frequently added to preservative solutions (1.5 to 4%) to increase the supply of respirable substrates to cut flowers and there-by improve the keeping quality (Rogers, 1973; Mastalerz, 1977; Nelson, 1978).

(c) Ethylene

Ethylene plays an important role in the development and senescence of flowers (Ables, 1973; Rogers, 1973). Fisher (1950) reported that a wide range of flowers produce ethylene in varying amounts. The quantity of ethylene produced may vary greatly during the post harvest life of the flower. A surge in ethylene production may accompany wilting of some flowers e.g. carnation (Nichols, 1966) but others such as chrysanthemum,

narcissus and anemone do not produce large volumes of ethylene during wilting (Nichols, 1966).

Flowers vary considerably in their sensitivity to ethylene and there are differences among varieties and betweenstages of physiological development. Orchids (Arditti *et al.*, 1973; Beyer, 1976), carnations (Smith *et al.*, 1966; Barden and Hanan, 1972; Maxie *et al.*, 1973; Mayak and Dilley, 1976) and snapdragons (Lumsden *et al.*, 1940; Farnham *et al.*, 1980) are some flowers that are very sensitive to low levels of ethylene. Work by Paull *et al.*, (1980) indicates that ethylene is not important in determining the vase life of *Protea*, but other members of the family *Proteaceae* including *T. speciosissima*, have not yet been examined.

A large number of chemicals have been shown to reduce or prevent ethylene synthesis in flowers (Table 2.2). Silver salts, e.g. silver thiosulphate, are widely used for the suppression of ethylene synthesis in flowers, especially carnations. Silver ions also reduce the sensitivity of the flower to exogenous ethylene. Extremely small amounts of silver thiosulphate cause a doubling of the vase life of carnations (Nichols, 1980).

(d) Temperature

Low temperature during storage of cut flowers decreases the rate of respiration, rate of ethylene production, and overall floral development (Fischer, 1950, 1953; Mayak and Halevy, 1971). By reducing the respiration rate the carbohydrate reserves in the cut flower are conserved, which helps to maintain quality and prolong vase life (Nelson, 1978). Flowers are generally stored at temperatures from -0.6 to 4.4°C (Nelson, 1978).

Table 2.2 Some chemicals which either inhibit ethylene synthesis in cut flowers or protect cut flowers from extraneous ethylene.

Chemical	Reference
aminooxyacetic acid	Fujino *et al.*, 1980
benzyl isothiocyanate	Parups, 1975
2:4 dichlorophenoxyacetic acid	Sacalis and Nichols, 1979
ethanol	Heins, 1980
silver ions	Veen, 1979 a, b
sodium benzoate	Baker *et al.*, 1977
sodium borohydride	Camprubi and Bargalla, 1980
sucrose	Mayak and Dilley, 1976
thiabendazole	Apelbaum and Katchansky, 1978

The length of time that flowers may be stored at low temperatures is variable. Opened roses may be held in cold store for up to 18 days and chrysanthemums and carnations for up to 21 days but gladioli may only be stored for much shorter periods (Nelson, 1978). Bud carnations (unopened flowers) may be held at low temperatures for up to 4 weeks (Kofranek *et al.*, 1972) or with the use of preservatives, fungicides and optimal environmental conditions, up to 24 weeks (Rudnicki, 1981).

In the family *Proteaceae, Leucospermum* flowers will store well for periods of up to 3 weeks at 20°C but the quality of *Protea compacta, P. repens* and *P. punctata* after 3 weeks storage is poor (Jacobs, 1982).

(e) Water quality

The quality of the water used in both holding solutions and vases can have a significant effect on the vase life of cut flowers. Tapwater may contain bacteria, organic matter and/or salts which may reduce the vase life of cut flowers (Rogers, 1973; Reid and Kofranek, 1980; Jacobs, 1982).

Jacobs (1982) recommended that deionised water be used for holding a range of *Proteaceae* inflorescences, but there appears to be no experimental basis for this recommendation. Growers in South Africa utilize either tapwater or borehole water for holding flowers of the *Proteaceae* (Jacobs, 1982). There is an obvious need to determine the effect of water quality in holding solutions on the post harvest life of *T. speciosissima* inflorescences.

(f) Micro-organisms

Cut flowers infected with micro-organisms will often have a reduced

vase life (Nelson, 1978). Roses with leaves infected with black spot fungi (Williamson, 1950) and chrysanthemum flowers infected by *Botrytis* (Dimock and Baker, 1950) have a reduced vase life, due principally to ethylene production by the damaged tissue. *Rhizopus* and *Botrytis* can develop on *Leucospermum* during storage at high relative humidities, even at low temperatures (2°C), causing a reduction in the visual quality of the flower and tissue breakdown. Dicloran and benomyl effectively suppress these diseases on *Leucospermum* for up to 2 weeks, but these fungicides did not protect *Protea* flowers stored at 6-11°C for 24 days (Jacobs 1982) nor carnations stored at low temperatures (0-1°C) (Kofranek *et al.*, 1972; Rudnicki, 1981).

(g) Conditions for measuring the vase life of cut flowers

Standardised environmental conditions should be used to assess treatments used to modify (usually extend) the vase life of cut flowers, so as to enable the comparison of results among various experimenters. Sytsema (1975) and Reid and Kofranek (1980) suggested that temperature, relative humidity, light and gas concentration (ethylene) can have a large effect on vase life. In addition, Sytsema (1975) listed conditioning of the flowers and Reid and Kofranek (1980) mentioned water quality as being another important factor.

Reid and Kofranek (1980) recommended a temperature of 20°C ($\pm$2°), a relative humidity of between 60 and 70%, a light intensity of 1,000 lux (cool white fluorescent tubes) and good ventilation (to prevent a build up of ethylene) for vase life assessments. These conditions are similar to those recommended by Sytsema (1975).

(h) Criteria for the evaluation of post harvest life of cut flowers

The goal for the extension of post harvest life of cut flowers is consumer satisfaction (Salinger, 1975). Of the factors considered by Salinger (1975) to be important in determining consumer satisfaction, those applicable to inflorescences of *Telopea speciosissima* are:-

(a) vase life

(b) flower development in the inflorescences

(c) retention of flower and bract colour

(d) maintenance of attractive foliage

(e) opening of buds after harvest

(f) retention of vase life after cool storage and transport

Vase life determines how long the producer, wholesaler and retailer may hold flowers and how long the consumer gains satisfaction from the flowers. The vase life of flowers ends when they lose most of their decorative value. This is, perhaps, the most important factor in evaluating new species (Parvin *et al.*, 1973; Watson and Parvin, 1973).

For most *Protea* the earliest harvest time is when the individual bracts at the apex of the inflorescence become loose. The latest harvest stage is when the bracts have opened to form a cylinder, well before the perianths split open to release the style. The optimum stage for harvesting *Leucospermum* which has a structure closer to that of *T. speciosissima,* is when three-quarters or more of the styles have reflexed (Jacobs, 1982).

There is an obvious need to determine:-

(a) the optimum stage for harvesting and

(b) a series of standards to characterise the stages in inflorescence

development to assess treatments for extending storage and vase life.

The availability of techniques for medium and long term storage is important to producers of *Proteaceae* in South Africa for both storage during periods of over-supply and for storage during transport to distant markets. Cold storage for 2-3 weeks can ease marketing problems encountered with *Leucospermum*, a species which has a peak of production in October in South Africa (Jacobs, 1982). Cold storage is more important for *T. speciosissima* because it has a much shorter flowering period (Parvin *et al.*, 1973). If *T. speciosissima* inflorescences could be stored for extended periods it might be possible to use sea transport to European markets instead of the more costly air freight (Jacobs, 1982; Larkman, 1982). Unfortunately, extended periods of storage generally reduced the vase life of cut flowers (Mastalerz, 1977; Nelson, 1978; Jacobs, 1982). Nevertheless there is a need to determine how storage affects the vase life of *T. speciosissima*.

PART 1

CHAPTER 3 VEGETATIVE PROPAGATION OF *TELOPEA SPECIOSISSIMA* (EXPERIMENT 1)

Introduction

Propagation of *T. speciosissima* from seed is not difficult. Seed is freely produced and, if fresh, germinates readily. However, large variation in vigour, flower shape and size, and leaf shape exists between individuals propagated from seed. Vegetative propagation would allow the multiplication of desirable individuals.

The use of auxins, fungicides, cytokinins and the physiological stage of the cutting material are all important factors in adventitious root formation on cuttings. This section deals with the effect of indolebutyric acid (IBA), benomyl which has both fungicidal and cytokinin-like properties (Skene, 1972), time of collection throughout the year and growing conditions on rooting of *T. speciosissima* cuttings.

Materials and Methods

Vegetative *T. speciosissima* cuttings were taken from Mount Wilson, Blue Mountains (33°61'S, 150°23'E) and Mangrove Mountain (33°19'S, 151°13'E) in the Central Coast area of New South Wales. At Mount Wilson (elevation 1066m; average summer maximum 23°C, minimum 12°C; average winter maximum 10°C, minimum 3°C) mother plants were growing in the wild in a dry sclerophyll forest. At Mangrove Mountain (elevation 274m; average summer maximum 24°C, minimum 14°C; average winter maximum 16°C, minimum 6°C) cuttings were taken from cultivated plants. These had received a total of 200 kg/ha superphosphate in the previous 2 years and received regular irrigations (Plate 1.2). All cuttings were from terminal portions of the stem and were trimmed to 20 cm of stem and 5-6 leaves. Those from Mangrove

Mountain were thinner than those from Mount Wilson (7 mm average compared to 10 mm respectively)

The experimental design was a 4 factor (incomplete) factorial. Treatments with cuttings from Mount Wilson had 2 replications of 10 cuttings, but due to a shortage of available material those from Mangrove Mountain were not replicated.

The treatments were:

(1) Source of cuttings: Mount Wilson and Mangrove Mountain sites.

(2) Sampling-time: Cuttings were taken at monthly intervals from March 1973 to February 1974. The condition and state of the terminal bud were noted for the majority of the cuttings at each sampling-time.

(3) Treatment with IBA: Cuttings were treated by the concentrated solution dip method (Hartman and Kester, 1968). The basal 5 mm of the cuttings were dipped in a 50% ethyl alcohol solution containing 0, 500, 1000, 2000 or 4000 ppm (w/v) of IBA for 5 seconds. The 4000 ppm IBA treatment was not included at the first 3 sampling times (March, April and May). This was responsible for the factorial being incomplete.

(4) Treatment with benomyl: Cuttings to be treated with benomyl were immersed in a 250 ppm (w/v) benomyl solution for 30 seconds after the IBA solution had dried. The control cuttings were immersed in water.

After treatment, the cuttings were planted in pots containing perlite and placed in a glasshouse (28°C maximum; 20°C miminum) under an intermittent mist on a sand bed heated to 28°C. The light intensity in the glasshouse was reduced by 60% with shade cloth.

After 4 weeks the cuttings were removed from the pots and the number

with roots longer than 5 mm counted. Those with no roots longer than 5mm were replaced and re-examined after a further 4 weeks.

Prior to statistical analysis as an incomplete factorial, an arcsin $\sqrt{x}$ transformation was applied to all percentage data. When x was 100% and 0%, 97.5% and 2.5% were substituted for x respectively. Steel and Torrie (1960) recommend this transformation to equalise variances for percentage data covering a wide range.

All root length data were calculated on an average root length per cutting basis. A $\sqrt{(x + 0.375)}$ transformation was applied to these data. Steel and Torrie (1960) recommended a $\sqrt{x}$ transformation to equalise variances for data consisting of small numbers. The 0.375 correction was applied because when very small values are involved the $\sqrt{x}$ transformation tends to over-correct and it is also essential when zeros are involved.

Results

After four weeks there was no significant difference between the 2 locations in the number of cuttings that had rooted ($P > 0.05$).

However, after 8 weeks, cuttings from Mangrove Mountain had a significantly higher ($P < 0.05$) cummulative percentage strike (51%)[1] than those from Mount Wilson (36%)[1]. There were no significant interactions involving source of cuttings.

Percentage rooting or mean root length per rooted cutting of benomyl-

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1. Untransformed means. However, statistical analysis was done with transformed data.

treated cuttings did not differ significantly from the control cuttings after 4 weeks. However, after 8 weeks, benomyl had significantly increased the cumulative percentage of rooted cuttings from 33%[1] to 58%[1]. Treated cuttings had only minor infections of Black Spot (*Guignardia citricarpa* Kiely). The identity of the disease organism was confirmed by T. Kiely (pers. comm.). In untreated cuttings the symptoms were far more severe; whole leaves became necrotic. There were no significant interactions involving the benomyl treatment dispite one site being surrounded by citrus infected with *Guignardia citricarpa* and the other being many kilometres removed from the nearest citrus trees.

Increasing the concentration of IBA significantly increased both the percentage of rooted cuttings (Figure 3.1) and the root length (Figure 3.2). After root initiation, the base of some cuttings blackened progressively both up the stem and along the roots. This effect was confined mostly to those treated with 4000 ppm IBA (Plate 3.1). Fifty cuttings were sectioned and examined for the presence of micro-organisms but none was identified.

During the winter months the mother plants were dormant except for the growth of flower buds. Vegetative growth and flowering in the spring began 1 month earlier at Mangrove Mountain than at Mount Wilson, possibly owing to the milder climate at Mangrove Mountain. Growth also ceased later in the autumn at Mangrove Mountain.

Time of year when cuttings were taken had a large effect on rooting. Cuttings collected in September and October from both locations produced the highest percentage of rooted cuttings (Figure 3.3) as well as the

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1. Untransformed means. However, statistical analysis was done with transformed data.

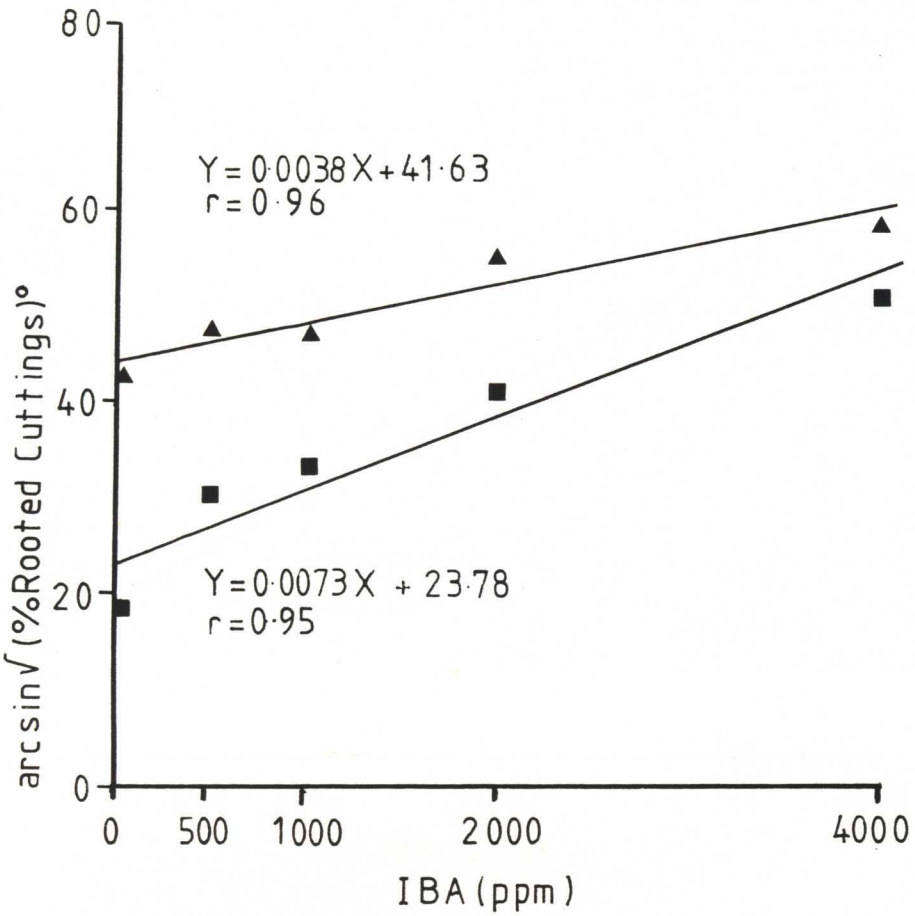

Figure 3.1 Effect of applied IBA concentration on the cumulative percentage of rooted cuttings after 4 (■) and 8 (▲) weeks.

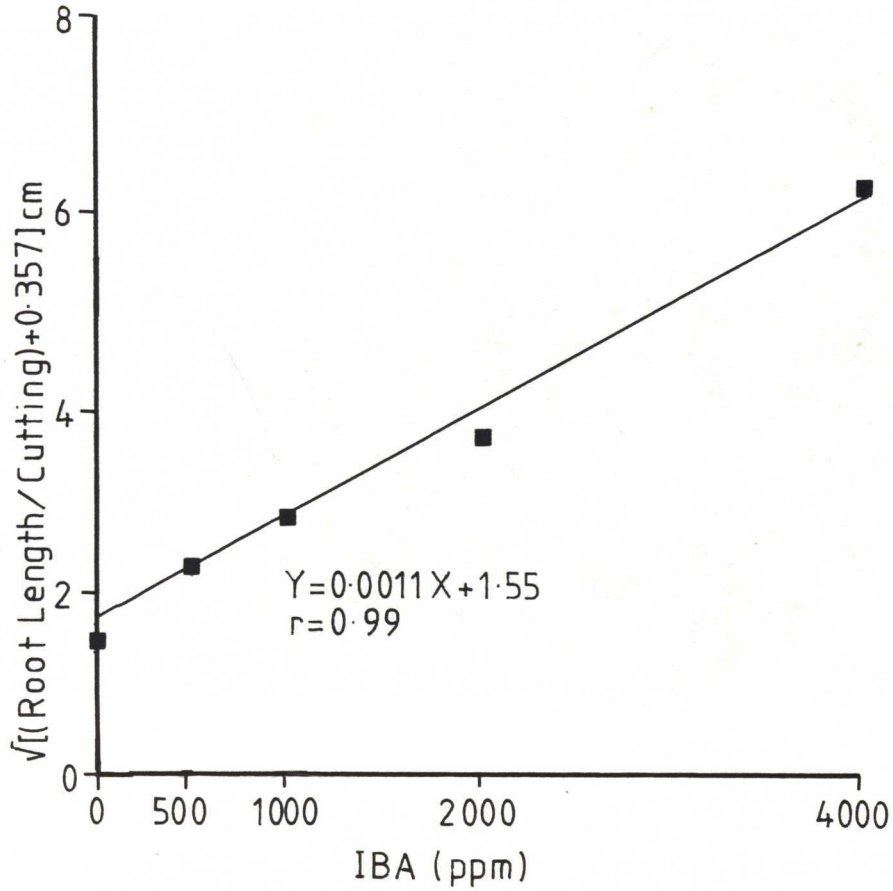

Figure 3.2 Effect of the concentration of IBA on the mean total root length (cm) per rooted cutting 4 weeks after collection.

Plate 3.1 Effect of Benomyl and IBA on rooting of cuttings of *Telopea speciosissima*. From left to right -

(a) not treated with benomyl and infected with *Guignardia citricarpa*.

(b) treated with 250 ppm benomyl and 0 ppm IBA.

(c) treated with 250 ppm benomyl and 500 ppm IBA

(d) treated with 250 ppm benomyl and 100 ppm IBA

(e) treated with 250 ppm benomyl and 4000 ppm IBA - basal rot began on this cutting after the formation of roots

(scale on left - 1 div. = 1 cm)

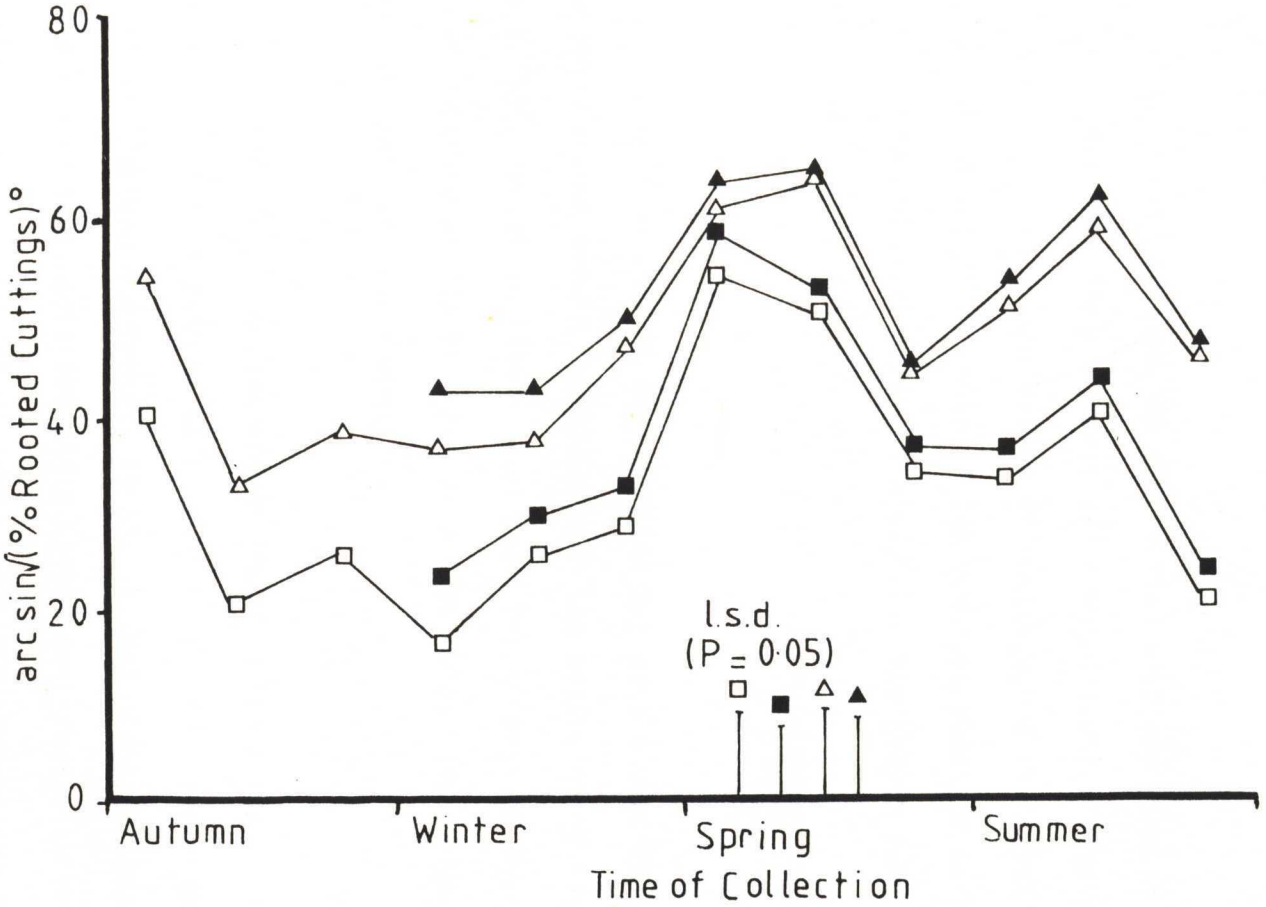

Figure 3.3 Effect of time of collection of cuttings and IBA concentration on the cumulative percentage of rooted cuttings. □ – average for 0-2000 ppm IBA 4 weeks after collection; ■ –average for 0-4000 ppm IBA 4 weeks after collection; △ – average for 0-2000 ppm IBA 8 weeks after collection; ▲ – average for 0-4000 ppm IBA 8 weeks after collection

greatest length of roots (Figure 3.4). This period corresponds with the beginning of vegetative growth in spring (Table 3.1). Another peak in root formation occurred in March just before vegetative growth ceased. The percentage of rooted cuttings and length of root formed was poor when the plants were vegetatively dormant (e.g. winter).

Response to applied IBA also varied with the time of year at which the cuttings were taken. There was a significant interaction ($P < 0.05$) between the month of collection of cuttings and the level of IBA (Table 3.2). The greatest response to IBA occurred when the mother plants were growing and was least during winter, when the growth rate was low as was the percentage of rooted cuttings.

Discussion

The optimum time to take cutting for both successful rooting and subsequent root growth coincided with the beginning of vegetative growth in spring. Rousseau (1967) also found for a range of *Proteaceae* that cuttings should be taken at a particular time of year for the greatest rate of root formation.

Growing conditions can affect the carbohydrate and other nutrient reserves of mother plants which can have a large effect on the rooting of cuttings (Hartman and Kester, 1975). Mother plants at Mangrove Mountain had a longer growing-period and a higher soil fertility than those at Mount Wilson. The better growing conditions at Mangrove Mountain may have been responsible for the 15% increase in the number of cuttings rooted from this area.

Since benomyl had no effect on either root length or on percentage

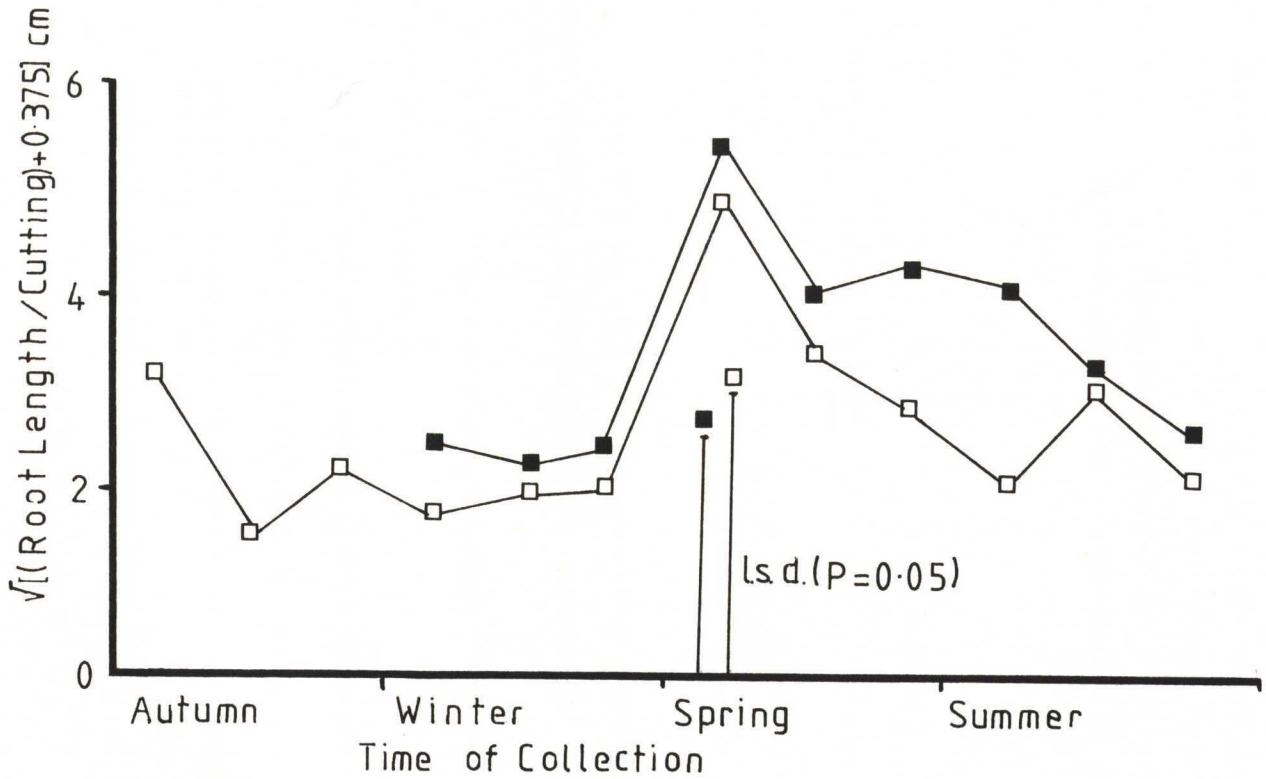

Figure 3.4 Effect of time of collection of cuttings and IBA concentration on the average root length per rooted cutting 4 weeks after collection.

□ – average for 0-2000 ppm IBA; ■ – average for 0-4000 ppm IBA.

Table 3.1 Condition of propagation material at the time of collection.

Month of collection		Source of cuttings	
		Mangrove Mountain	Mount Wilson
Autumn	March	D	D
	April	A	A
	May	A	A
Winter	June	A	A
	July	A	A
	August	A	A
Spring	September	B*	A
	October	C*	B*
	November	C	C*
Summer	December	D	C
	January	D	D
	February	D	D

Key (for the majority of the propagation material at any one collection time)

A - stems lignified and leaves mature. Vegetative terminal buds dormant

B - as A but new growth emerging from vegetative terminal bud

C - approximately half of cutting is new seasons growth

D - Entire cutting new seasons growth. Stems lignifying, leaves emerging from terminal bud

* - mother plant flowering

TABLE 3.2

Effect of month of collection of cuttings and level of IBA on the cumulative percentage of rooted cuttings 4 and 8 weeks after collection.

MONTH OF COLLECTION		Transformed Data (degrees)									
		4 weeks after Collection Level of IBA (ppm)					8 weeks after Collection Level of IBA (ppm)				
		0	500	1000	2000	4000	0	500	1000	2000	4000
Autumn	March	20	43	44	56		39	62	58	65	
	April	13	20	21	29		20	31	37	39	
	May	13	23	25	38		21	42	37	52	
Winter	June	13	13	18	21	51	22	32	43	50	63
	July	13	20	23	42	48	22	33	33	51	62
	August	13	21	26	42	51	35	37	45	62	63
Spring	September	31	50	55	71	77	39	56	66	76	78
	October	21	62	48	60	58	52	72	57	67	63
	November	15	35	37	39	41	23	49	48	45	47
Summer	December	19	34	33	30	49	42	54	48	48	58
	January	27	36	44	40	51	54	56	58	53	61
	February	15	13	22	22	30	41	44	41	48	48

lsd (P = 0.05) = 15
lsd (P = 0.01) = 20

lsd (P = 0.05) = 16
lsd (P = 0.01) = 22

cuttings rooted after 4 weeks, there is no evidence that it acted as a cytokinin. The 25% increase in the percentage of rooted cuttings after 8 weeks is likely to have resulted from its fungicidal properties, especially its ability to suppress *G. citricarpa* (Kiely, 1971).

In general the more concentrated the solution of IBA applied, the higher the percentage of rooted cuttings and the greater the root length. A large number of rooted cuttings, however, subsequently died, in particular those treated with 4000 ppm IBA. Since a pathogen could not be associated with the deaths, there may have been a delayed toxicity to IBA, such as that exhibited by *Mandevilla* and some species of *Ficus* (Hartman and Kester, 1968).

When delayed toxicity to IBA is taken into account, the optimum level of IBA in this experiment was 2000 ppm, even though at this level some delayed toxicity to IBA still occurred. However, the response of the cuttings to IBA varied with the season. When the mother plant was actively growing such as in early spring, the cuttings responded better to low levels of IBA than when the plant was dormant, such as in mid-winter. The differing response of some species to exogenous cytokinins and auxins has been attributed to changes in the endogenous levels of the cytokinin : auxin ratio during the growing season (Wareing, 1973 ; MacGuire and Flock, 1975). Thus, a possible reason for the poor response of *T. speciosissima* to IBA during the winter is the limiting factor of low endogenous levels of cytokinins.

PART II

MINERAL NUTRITION OF *TELOPEA SPECIOSISSIMA*

CHAPTER 4: THE EFFECT OF NITROGEN SOURCE ON THE GROWTH OF *T. SPECIOSISSIMA* (EXPERIMENT 2)

Introduction

Source of N in a potting medium solution can have a large effect on its pH (Hewitt, 1966). If NO_3^- is used as the N source the pH of the media will rise due to the differential uptake of NO_3^- and cations. Use of NH_4^+ as the N source will cause the pH to fall, again due to differential uptake (Hewitt, 1966; Bunt, 1976). Control of pH is important because plants may have different optima for uptake of NO_3^- and NH_4^+ ions. Uptake of other ions will also be affected. Generally maximum absorption of NH_4^+ takes place at a pH of 6 or above, whereas uptake of NO_3^- is either facilitated by a slightly acid reaction (4.5 to 6) or is independent of pH (Hewitt, 1966). Different N sources caused the pH of the medium solution used for growing *Eucalyptus* to vary between 4.25 and 8.75 where the media solution was not replaced (Moore and Keraitis, 1971). Despite the importance of pH on the uptake of various ions, it was not controlled in numerous experiments reported in the literature on the nutrition of Australian native plants and members of the family *Proteaceae* (Specht and Groves, 1966; Moore and Keraitis, 1971; Grundon, 1972; Nichols and Beardsell, 1981, Claassens and Folscher, 1982).

When the ratio of NO_3^- to NH_4^+ is varied in the nutrient solution used for sand culture, the proportion of other cations and/or anions (balancing ions) must also be varied, confounding any effect of the

NO_3^- : NH_4^+ ratio. Sulphate is often chosen as a balancing ion because of its low toxicity to plants (Hewitt, 1966; Moore and Keraitis, 1971; Bunt, 1976; Nichols and Beardsell, 1981 ; Claassens and Folscher, 1982) Chloride and Na^+ ions were also used by some investigators because of their limited role in plant nutrition (Hewitt, 1966; Moore and Keraitis, 1966; Bunt, 1976; Specht and Groves, 1966; van Staden, 1968; Groves and Keraitis, 1976). Often little or no attempt has been made to distinguish between the effects of the nutrient under investigation and the balancing ions used.

The confounding effect of the balancing ions may be partly overcome by use of different balancing ions as mutual controls (e.g. the use of both Cl^- and SO_4^{2-}) and tissue analysis (Hewitt, 1966).

Experiment 2 examines the effect of N source on the growth of *T. speciossissima* (a) when the nutrient solution was not changed (static situation), and (b) when the nutrient solution is changed daily.

EXPERIMENT 2 (a) : The effect of nitrogen source on the growth of *T. speciosissima* in a static situation.

Materials and Methods

Five *T. speciosissima* seeds[1] were sown in each of 80, 12.7 cm drained pots containing 100 g of perlite, in a glasshouse with 60% shade and a maximum day temperature of 30°C. Seedlings were thinned after 4 weeks to 2 per pot to obtain a high degree of uniformity. A total of 1 litre of a modified Hoagland's solution (Arnon, 1938) was applied in 20 equal applications of 50 ml each, 3 times a week, beginning when the seedlings were thinned. The solution contained 6.0 mM K^+, 6.0 mM SO_4^{2+},

1. from Somersby, N.S.W.

5.0 mM Cl^-, 4.0 mM Ca^{2+}, 2.0 mM Mg^{2+}, 1.0 mM PO_4^{3-} and trace elements at the rate used in Hoagland's solution. The solution was made up using analytical reagents and de-ionised water and adjusted to a pH of 5.5 with H_2SO_4 before application. De-ionised water was also added to the pots when required. Leaching was prevented by placing plastic saucers under each pot; excess water was re-absorbed as the perlite dried out.

The ratios of N sources applied are given in Table 4.1. Half was applied at thinning and the remainder a week later.

The design of the experiment was completely randomised with 16 replicate pots for treatments 1 and 5, or 8 replicates for the other treatments (2A, 2B, 3A, 3B, 4A, 4 B) each with two sub-samples. Total shoot dry weight of the plants and pH of the media (1:2 - perlite: water, w/w) was measured at harvest (8 weeks after thinning). Results were analysed as a one factor analysis of variance.

Results

Nitrogen source had a significant effect on the shoot dry weight of *T. speciosissima*. Greatest growth occurred where 50% of the N was applied as NO_3^- and 50% as NH_4^+ (Figure 4.1, Plate 4.1). Where the N was applied as all NO_3^- (treatment 1)plants were chlorotic (Plate 4.2) and 6 of the 16 plants in this treatment died. In the other treatments no plants were chlorotic or died. Plant dry weight was reduced when N was applied as NH_4Cl (treatment 5). At the levels applied, additional Cl^- and/or Na^+ (2A vs 2B, 3A vs 3B, 4A vs 4B) had no significant effect ($P > 0.05$) on the shoot weight of plants. The pH of the media (Figure 4.1) at the conclusion of the experiment varied from 7.65 (treatment 1 - all NO_3^-) to 4.51 (treatment 5 - all NH_4^+). Additional Cl^- and/or Na^+ had no significant effect ($P > 0.05$) on the pH of the media.

Table 4.1 Fertilizer treatments used in the Experiment 2(a) - Effect of the source of nitrogen on the growth of *T. speciosissima* in a static situation

Treatment No.	NO_3^-:NH_4^+ ratio	Final applied ion concentration (*m* moles/pot)			
		NO_3^-	NH_4^+	Na^+	Cl^-
1	4:0	15.00	-	15.00	-
2A	3:1	11.25	3.75	7.50	-
2B	3:1	11.25	3.75	11.25	3.75
3A	2:2	7.50	7.50	-	-
3B	2:2	7.50	7.50	7.50	7.50
4A	1:3	3.75	11.25	-	7.50
4B	1:3	3.75	11.25	3.75	11.25
5	0:4	-	15.00	-	15.00

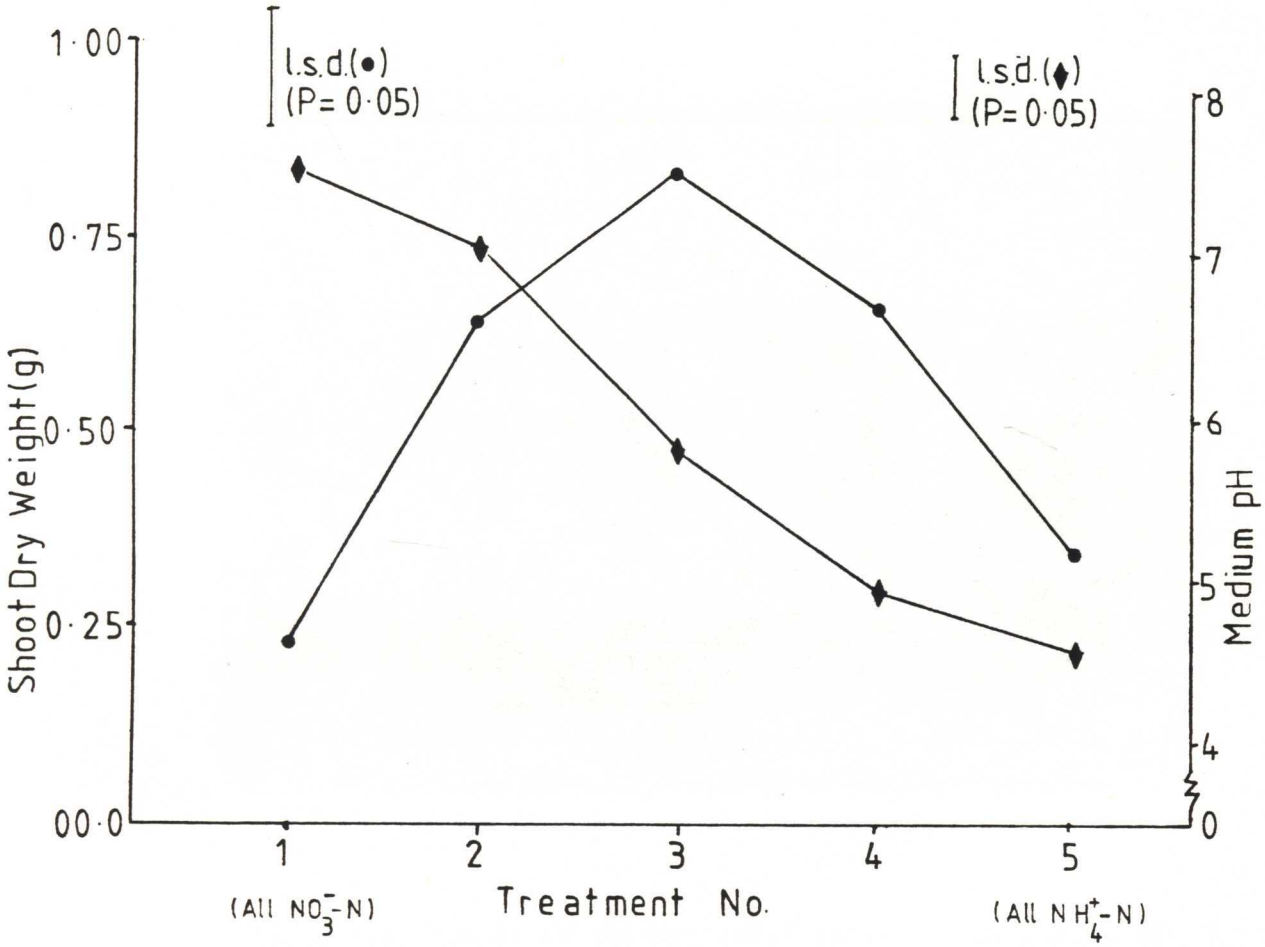

Figure 4.1 Effect of N source on the shoot dry weight (●) and medium pH (♦) of *T. speciosissima* grown in perlite. Shoot dry weight l.s.d. only applies to treatments 2 - 5 due to deaths in treatment 1.

Plate 4.1 Effect of the NO_3^-:NH_4^+ ratio on the growth of *T. speciosissima*. From left to right 4:0, 3:1, 2:2, 1:3 and 0:4.

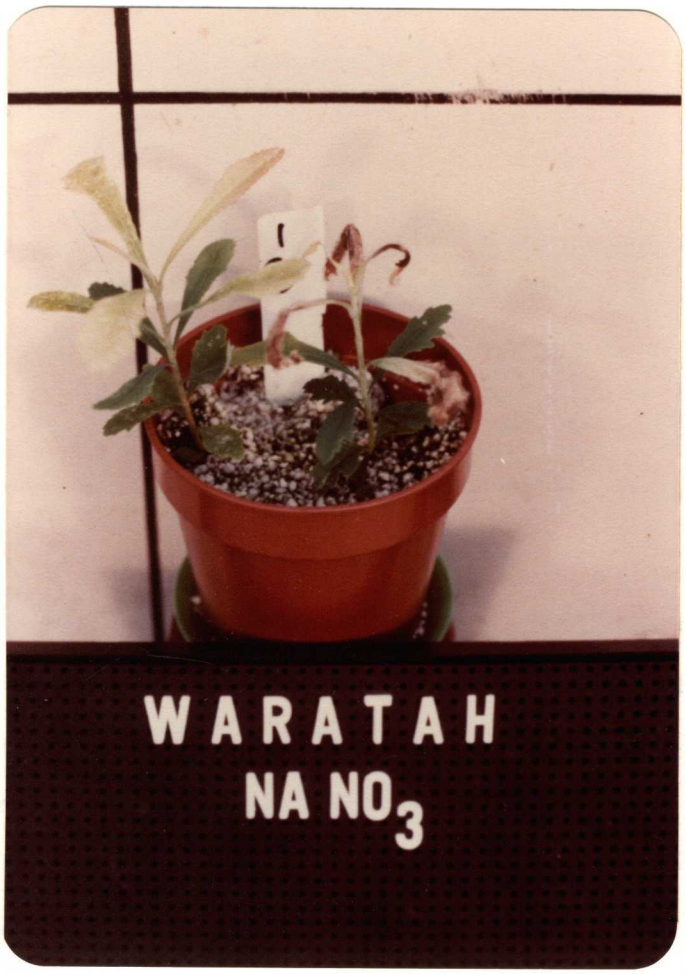

Plate 4.2 Effect of the all NO_3^- treatment on seedlings of *T. speciosissima*

EXPERIMENT 2 (b): Effect of nitrogen source on the growth of *T. speciosissima* with daily application of nutrient solutions.

Materials and Methods

(a) General

Five week-old seedlings[1] of *T. speciosissima* previously grown in perlite and distilled water were transplanted, one to a pot, into 100 mm drained plastic pots containing 50g of perlite. The seeds had a mean initial dry weight of 58.4mg (s.e. =0.2). After irrigation and draining, each pot contained approximately 50g of water. The plants were grown in a glasshouse with 60% shade, minimum night temperature of 18°C, maximum day temperature of 25°C with supplementary fluorescent light (daylight type - 40 μ moles m^{-2} s^{-1}, 12 hour cycle) for six weeks. Plants were watered daily with 50ml of a modified 10% Hoagland's solution (Arnon, 1938) containing 1.5 mM N (as various NO_3^- : NH_4^+ ratios), 0.1 mM P (as PO_4^{3-}), 1.1 mM K^+, 0.4 mM Ca^{2+}, 0.2 mM Mg^{2+}, 0.3 to 1.8 mM S (as SO_4^{2-} excluding H_2SO_4 used for pH adjustment), 0.0 to 3.0 mM Cl^- (excluding HCl used for pH adjustment) and trace elements at 10% of the rate used by Hoagland. All solutions were initially adjusted to a pH of 5.5 with either H_2SO_4 or HCl. Analytical grade reagents and distilled water were used to prepare all solutions.

(b) Treatments

Plants were irrigated with one of five different solutions (not including different balancing ions) all containing the same N level (1.5 mM) but with various ratios of NO_3^- : NH_4^+. Treatments are presented in Table 4.2. Either SO_4^{2-} or Cl^- was used to replace the NO_3^- and provide a balancing ion to the NH_4^+ as its level increased. A total of 32 pots were irrigated with solutions at each ratio i.e. 16 replicates

1. from Somersby, N.S.W.

Table 4.2 Fertilizer treatments used in Experiment 2b - Effect of nitrogen source on the growth of *T. speciossisima* with daily application of nutrient solutions.

Treatment No.	NO_3^-:NH_4^+ ratio	Balancing ion used	Concentration of nutrients in the irrigation solution (mM)			
			NO_3^-	NH_4^+	SO_4^{2-}(1)	Cl^-
1	4:0	-	1.500	-	-	-
2A	3:1	SO_4^{2-}	1.125	0.375	0.375	-
2B	3:1	Cl^-	1.125	0.375	-	0.75
3A	2:2	SO_4^{2-}	0.750	0.750	0.750	-
3B	2:2	Cl^-	0.750	0.750	-	1.50
4A	1:3	SO_4^{2-}	0.375	1.125	1.125	-
4B	1:3	Cl^-	0.375	1.125	-	2.25
5A	0:4	SO_4^{2-}	-	1.500	1.500	-
5B	0:4	Cl^-	-	1.500	-	3.00

(1) In addition to the basal level used. Additional SO_4^{2-} (and Cl^-), besides that required to balance the NH_4^+, is also required to compensate for the loss of NO_3^- from the solutions.

per treatment except for treatment 1 which had 32 replicates.

Once a week, following watering, the first 20 ml of leachate were collected from each pot and its pH determined. The pH of the applied solution was then adjusted (by an equal but opposite amount) to compensate for any change in the pH of the leachate. The pH of solutions balanced with SO_4^{2-} and Cl^- were adjusted with H_2SO_4 and HCl respectively. Leachate data was first analysed as a three factor (N ratio x balancing ion x time) analysis of variance with treatment 1 omitted then analysed as a two factor (N ratio x time) analysis of variance with the balancing ions (A + B) pooled and treatment 1 included (Steel and Torrie, 1960).

(c) Measurements at Harvest

Harvest times and the number of replicates harvested at each time are given in Table 4.3.

(1) Plant measurements

Leaf area was determined on fresh leaves with a LI-COR photo-electric area meter. The presence or absence of proteoid roots was noted. Dry weights of the leaf, stem and roots were determined after drying at 80^0C for 48 hours. Dry weights and leaf area data were first analysed as a two factor (ratio of N sources x balancing ion) analysis of variance with treatment 1 being ignored. They were then analysed as a one factor analysis of variance with treatments A + B being pooled and treatment 1 included. Orthogonal coefficients were used to test for first and higher order polynomials (Steel and Torrie, 1960). Data for the presence of proteoid roots were analysed in a similar fashion to the dry weight data, except that an arc-sin $\sqrt{x}$ transformation (with appropriate substitutions for 0 and 100%)and a theoretical

Table 4.3 Harvest times and number of replicates harvested at the conclusion of Experiment 2b

Time after the final irrigation (hrs)	No. replicates harvested per treatment	
	Treatment 1 (4:0)	Other Treatments
1.00	4	2
2.00	4	2
3.00	4	2
4.25	4	2
5.75	4	2
7.50	4	2
25.00	8	4

variance was used (Steel and Torrie, 1960). Because of the limited material available, shoots (leaf + stem) for each of the 9 treatments were bulked for chemical analysis. After grinding and compression into tablets the Ca, K, S, Cl and P content of the shoots was determined by x-ray emission spectrometry (McLachlan and Crawford, 1970). Nitrogen was determined after Kjeldahl digestion with an Orion 95-10 ammonia-specific ion electrode (Anon, 1975). Regression analysis was used to statistically analyse Ca, K, S, Cl and P content data (Steel and Torrie, 1960).

(ii) Media measurements

After the plants were removed the perlite from each plot was mixed with water to give a total weight (water plus perlite) of 200 g. The mixture was then filtered and the pH of the filtrate determined. The NO_3^- concentration of the filtrate was determined directly with an Orion 93-07 nitrate-specific ion electrode (Barker, 1974). NH_4^+ concentration in the filtrate was determined with an Orion 95-10 ammonia-specific ion electrode after adjustment to a pH of 11 and NO_2^- with an Orion 95-46 specific ion electrode after acidification to pH 2.5 (Anon, 1975). The NH_4^+, NO_3^- and NO_2^- contents were expressed in terms of the original media solution (i.e. 50 g water per pot) with a correction being made for the additional water used for extraction. Regression analysis was used to statistically analyse media data at harvest (Steel and Torrie, 1960).

Results

(a) Plant growth

Varying the NO_3^- : NH_4^+ ratio significantly affected ($P < 0.05$) leaf area, leaf dry weight, stem dry weight and root dry weight. First and second order polynomials (but not 3rd order) were significant

($P < 0.05$). Equations are presented in Figures 4.2 and 4.3. The maximum dry weight response for the aerial portions of the plant occurred at a higher NH_4^+ concentration than that for the root weight.

Increasing the level of NH_4^+ in the irrigation solution to 1.125mM (NO_3^- : NH_4^+ - 1:3) or above, completely inhibited proteoid root growth (Table 4.4). There was no significant difference ($P < 0.05$) between the growth of plants where SO_4^{2-} or Cl^- had been used as a balancing ion nor was any interaction significant ($P > 0.05$)

(b) Chemical analysis of shoots

Increasing the NH_4^+ content of the irrigation solution significantly increased the percentage of N in the shoot ($P < 0.05$; Figure 4.4). There was no significant difference ($P > 0.05$) in the shoot tissue percentage N between plants supplied with NH_4^+ as $(NH_4)_2 SO_4$ or $NH_4 Cl$.

Increasing the level of Cl^- in the irrigation solution significantly ($P < 0.05$) increased both the percentage of Cl and to a lesser extent S in the leaves and stems (Figure 4.5). Increasing the level of SO_4^{2-} in the irrigation solution significantly increased the percentage of S, but not Cl in the leaves and stems (Figure 4.5).

The ratio of NO_3^- : NH_4^+ or level of Cl^- or SO_4^{2-} in the irrigation solution did not significantly affect ($P > 0.05$) the percentage of Ca (mean = 0.66, s.e. = 0.04), P (mean = 0.31, s.e. = 0.03) or K (mean = 1.11, s.e. = 0.10) in the shoot.

(c) pH and nitrogen source in the media

Variation in pH between treatments was small for the first two weeks but after this rapidly increased, especially in the

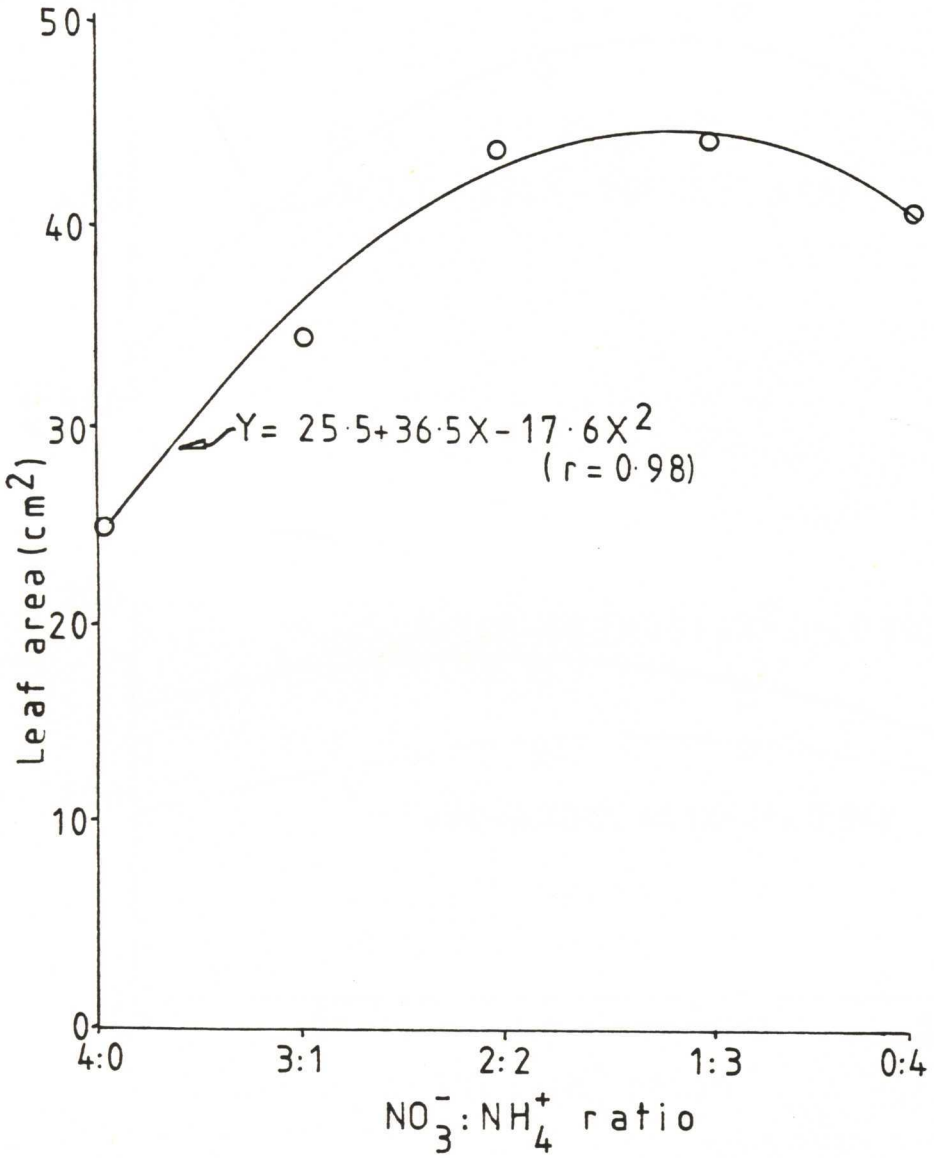

Figure 4.2. Effect of the NO_3^-:NH_4^+ irrigation solution ratio on the leaf area of *T. speciosissima*. The equation is expressed in terms of mM NH_4^+ (1.5 mM total N).

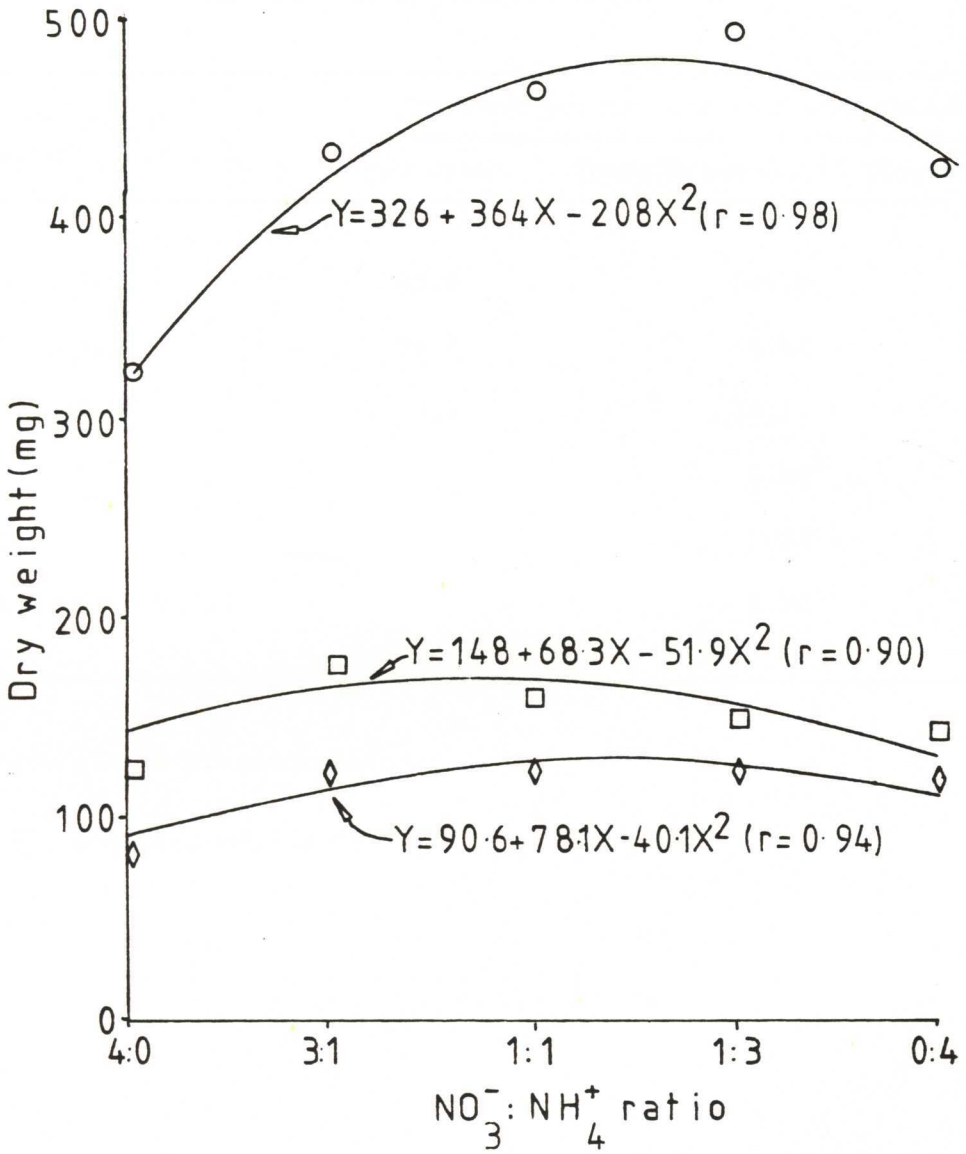

Figure 4.3. Effect of the NO_3^-:NH_4^+ irrigation solution ratio (1.5 mM total N) on the dry weight of leaves (○), roots (□) and stems (◊) of *T. speciosissima*. Equations are expressed in terms of mM NH_4^+.

Table 4.4 Effect of the NO_3^-:NH_4^+ ratio in the irrigation solution on the percentage of *T. speciosissima* seedlings with proteoid roots

Treatment (NO_3^-:NH_4^+ ratio)	Percent plants with proteoid roots	
	Raw data	Transformed data[1] (degrees)
4:0	65.6	54.10
3:1	56.3	48.60
2:2	3.1	10.14
1:3	0.0	0.80[2]
0:4	0.0	0.80
l.s.d. (P = 0.05)	-	2.50

[1] arc sin $\sqrt{x}$ transformation

[2] $\frac{25^o}{n}$ substituted for 0%

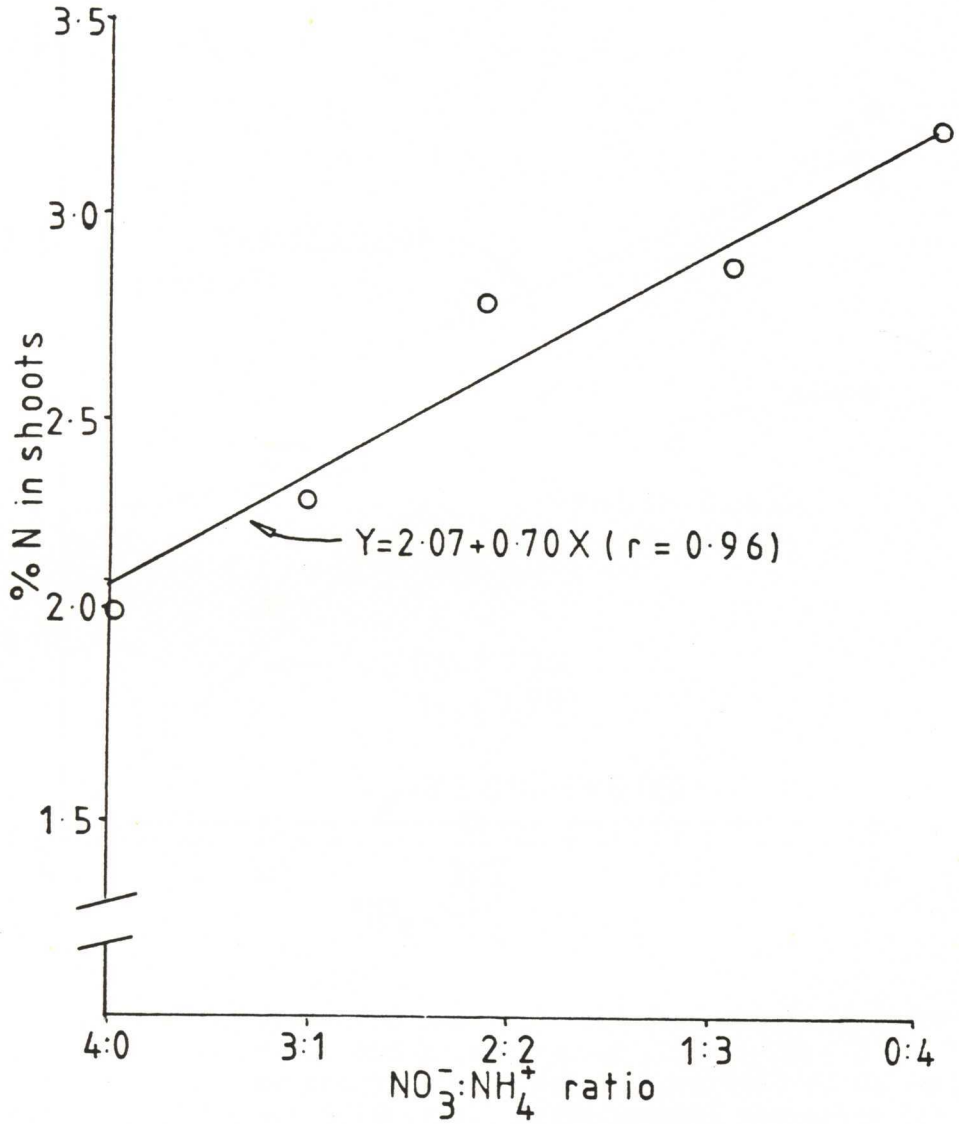

Figure 4.4. Effect of the NO_3^-:NH_4^+ irrigation solution ratio on the percentage of N in the shoots (dry wt.). The equation is expressed in terms of mM NH_4^+ (1.5 mM total N).

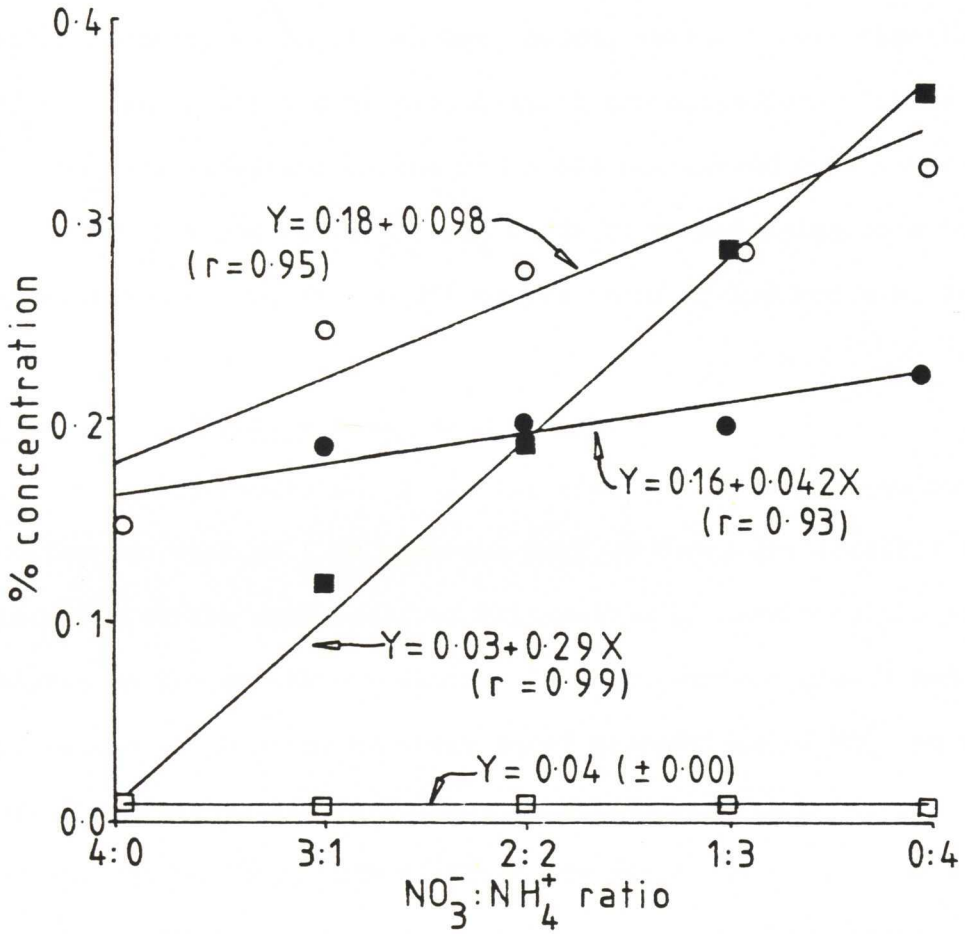

Figure 4.5. Effect of the NO_3^-:NH_4^+ irrigation solution ratio and balancing ion (Cl^- or SO_4^{2-}) on shoot concentrations (dry wt.) of Cl (■, □) and S (●, ○). Solid symbols represent Cl^- as a balancing ion and open symbols SO_4^{2-}. Equations are expressed in terms of mM NH_4^+ (1.5 mM total N).

treatments with the greater the NH_4^+ concentration in the irrigation solution. The leachate became more acid as the concentration of NH_4^+ increased (Table 4.5).

In the 25 hour period after the last irrigation, pH varied directly with NH_4^+ concentration. Both fell towards an equilibrium level (Figures 4.6 and 4.7). Although the NO_3^- concentration fell in all treatments where it had been added, this was only significant ($P < 0.05$) in the two highest initial concentrations (Figure 4.8). The NO_2^- concentration in the media did not exceed 0.07 mM at any time.

The use of $(NH_4)_2 SO_4$ vs NH_4Cl as balancing ions had no significant effect ($P > 0.05$) on the pH of either media or leachate.

Discussion (Experiments 2 (a) and 2 (b)

In Experiment 2 (a) the effect of N source was completely confounded with pH. As with the work of Moore and Keraitis (1971) this limits the usefulness of this method of examining the effect of N source on the growth of plants. However, maximum growth and minimal variation in pH occurred where equal proportions of NO_3^- and NH_4^+ were used (treatments 3A, 3B). Thus in any experiment to examine the effect of N level on the growth of *T.speciosissima*, where nutrients are not replaced, NH_4NO_3 may be used as the N source without other confounding ions or large variations in pH. The chlorosis that occurred in the all NO_3^- treatment in Experiment 2 (a) was probably due to the high pH restricting the uptake of Fe by the plant (Hewitt, 1966).

Experiment 2 (a) also demonstrated that additional NaCl and Na^+ or Cl^- at the rates used has no significant effect ($P > 0.05$) on the growth of *T. speciosissima* seedlings nor did substitution of SO_4^{2-} with Cl^- have a significant effect on ($P > 0.05$) on the growth of the

Table 4.5 Effect of NO_3^-:NH_4^+ ratio in the irrigation solution on the pH of leachate from pots

Treatment (NO_3^-:NH_4^+ ratio)	Time after commencement of treatments (weeks)					
	1	2	3	4	5	6 (1)
4:0	5.6	5.6	5.5	5.6	5.7	5.6
3:1	5.6	5.5	5.4	5.4	5.5	5.4
2:2	5.4	5.5	5.4	5.4	5.3	5.1
1:3	5.5	5.5	5.2	5.2	5.0	4.9
0:4	5.4	5.4	5.2	4.9	4.6	4.5

l.s.d. (P = 0.05) for weeks 1-5 = 0.3

(1) media pH 25 hours after final irrigation

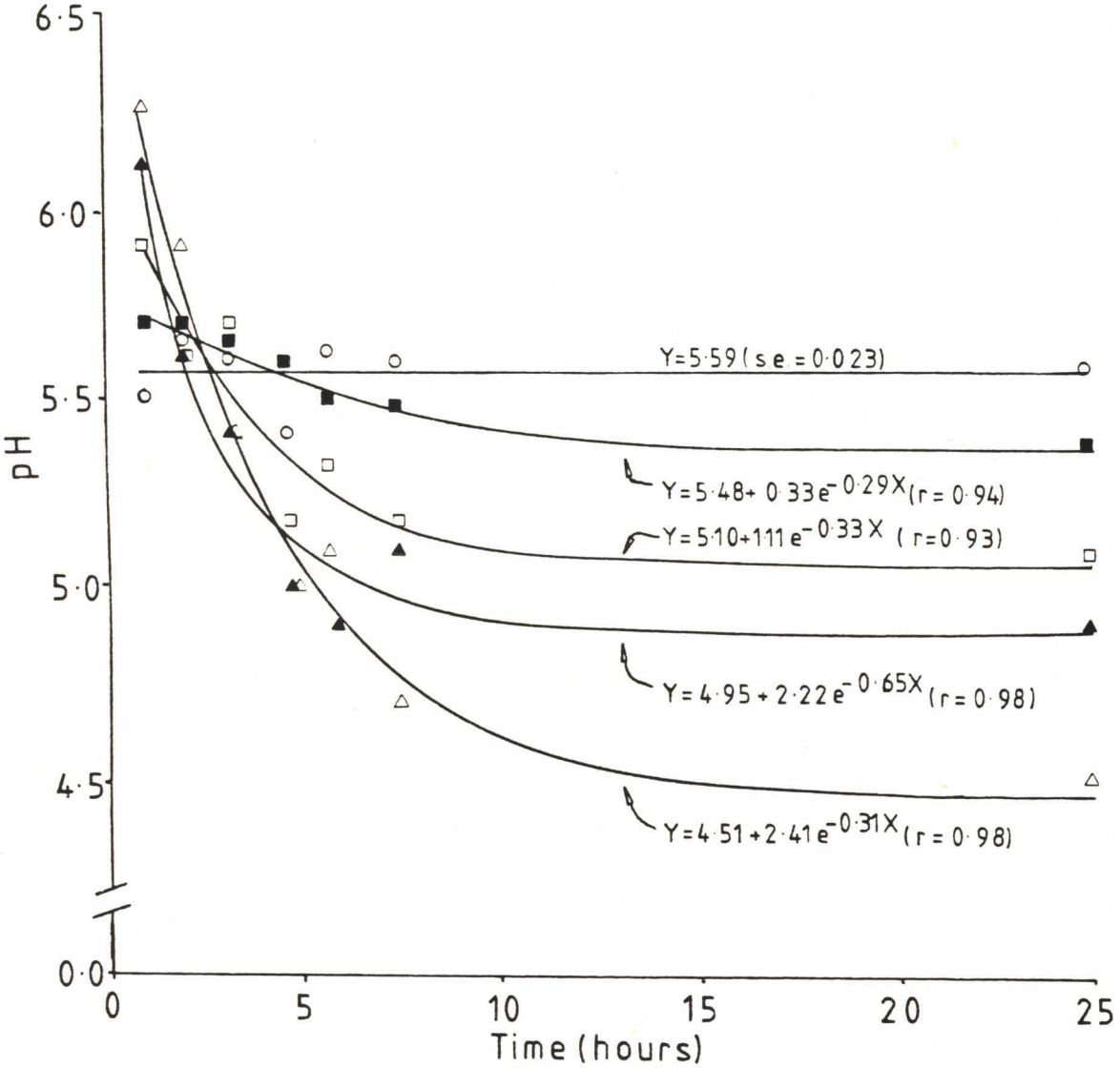

Figure 4.6. Effect of time after the final irrigation and NO_3^-: NH_4^+ irrigation solution ratio on the pH of the media solution. Ratios of NO_3^-:NH_4^+ - 4:0-o, 3:1-■, 2:2-□, 1:3-▲, 0:4-△.

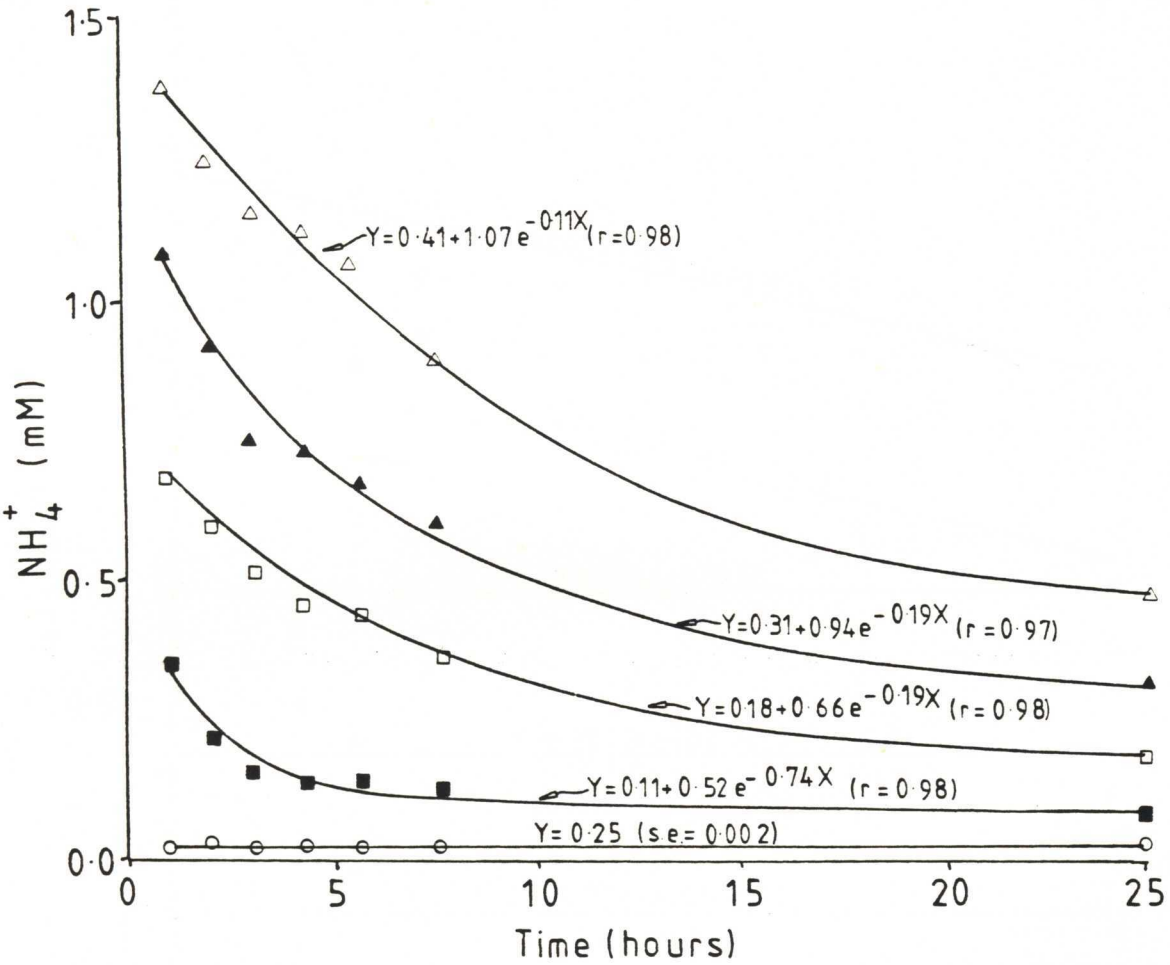

Figure 4.7. Effect of time after the final irrigation and NO_3^-: NH_4^+ irrigation solution ratio on the NH_4^+ concentration of the media solution. Ratios of NO_3^-:NH_4^+ - 4:0-o, 3:1- ■, 2:2- □, 1:3- ▲, 0:4- △.

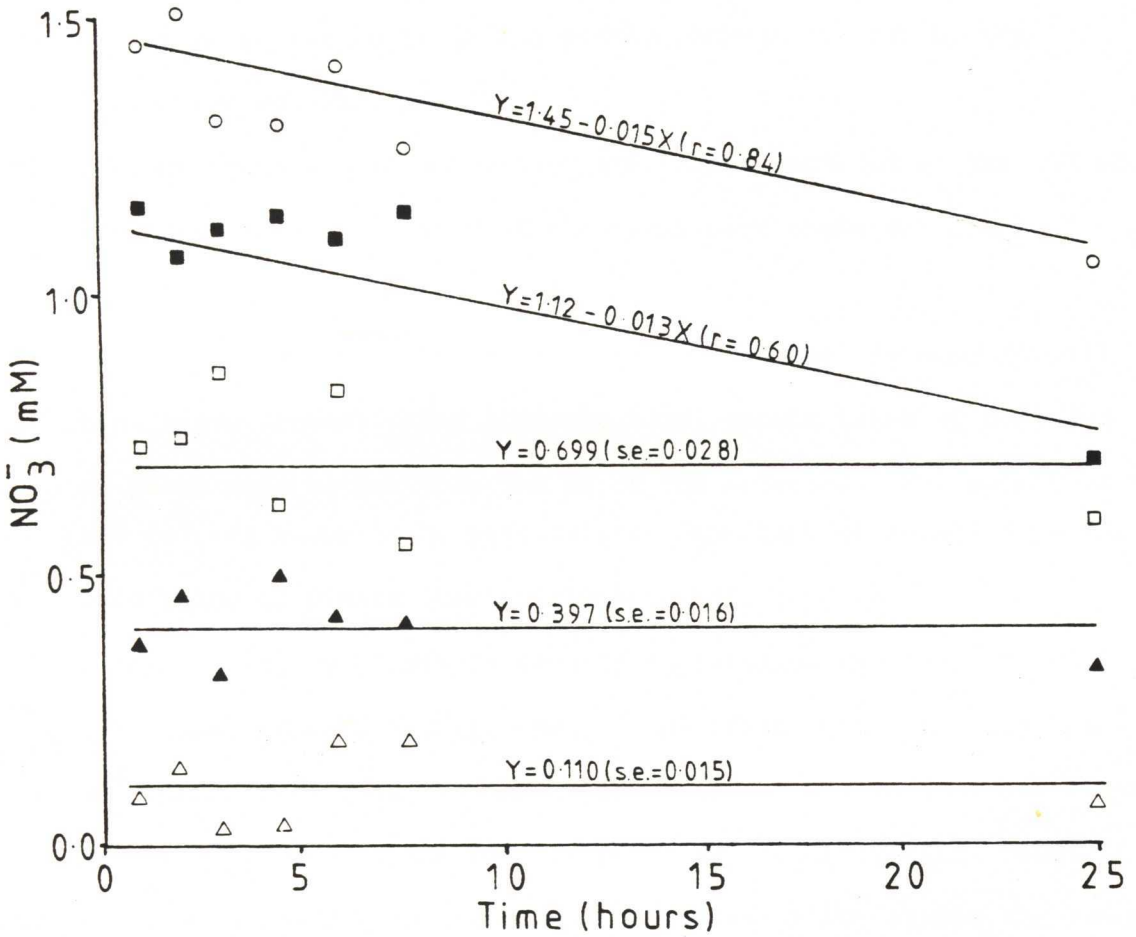

Figure 4.8 Effect of time after the final irrigation and NO_3^-: NH_4^+ irrigation solution ratio on the NO_3^- concentration of the media solution. Ratios of NO_3^-:NH_4^+ - 4:0-o, 3:1-■, 2:2-□, 1:3-▲, 0:4-△.

plants in Experiment 2 (b), despite tissue levels of S and Cl increasing with higher levels of SO_4^{2} and Cl^- respectively in the media. Thus addition of Na^+, Cl^- or SO_4^{2-} to the media at the levels used in Experiments 2 (a) and 2 (b) is likely to be of little biological significance to *T. speciosissima*.

Variation in media pH in Experiment 2 (b) probably had little effect on the growth of the seedlings because:-

(a) variation in the media pH was partly compensated for by the irrigation solution pH.

(b) maximum variation in pH between treatments occurred at the end of the Experiment. For most of the Experiment there was little variation in pH.

(c) the percentage of K, P and Ca in the shoots was the same for all treatments demonstrating that the total amount taken up depended on plant size rather than the pH of the solution. The uptake of K^+ and Ca^{2+} is known to be particularly dependant on solution pH for a wide range of plants (Hewitt, 1966).

(d) uptake of NH_4^+ by plants is usually facilitated by higher pH's i.e. (i.e. pH 6-7; Hewitt, 1966). The effect of increasing the NH_4^+ concentration in the irrigation solution was to increase tissue levels of N despite the fall in pH as NH_4^+ concentration rose.

(e) pH usually has little effect on the uptake of NO_3^- within the range 4.5-6 (Hewitt, 1966).

(f) the drop in pH at the higher NH_4^+ concentrations is unlikely to have been responsible for the inhibition of proteoid roots that occurred as they are normally formed in soils with a pH of about 4.5.

Maximum growth also occurred in Experiment 2 (b) when N was supplied in approximately equal proportions of NO_3^- and NH_4^+. Loss of NH_4^+ from the media was probably caused by uptake by the plant rather than

by microbial oxidation. The NO_3^- in the media did not increase and NO_2^- was not detected during the 25 hour period after the last irrigation in the all NH_4^+ treatment. Uptake of NH_4^+ was much greater than NO_3^- especially if the contribution of seed reserves of N are taken into account.

Published values for the N% in the seed (dry weight) of Australian native *Proteaceae* with a similar seed size and structure to *T. speciosissima* ranges from 10.5 to 13.8% (Grundon, 1972; Groves and Keraitis, 1976) with an average value for the species quoted by these authors of 11.75%. On a pro-rata basis, if *T. speciosissima* had a similar N content it would have reserves of approximately 0.50 m moles of N per seed which would account for approximately 60% of the N content of *T. speciosissima* grown in the all NO_3^- treatment (assuming no losses) compared to 27-32% of the total N content where 50% or more of the N was supplied as NH_4^+. Thus, although NO_3^- is taken up to some extent, NH_4^+ is preferred by *T. speciosissima* as is the case with a number of other species of the family *Proteaceae* (Claassens and Folscher, 1982).

The rate of removal of NH_4^+ from the media solution for treatments at the termination of Experiment (b) with a NO_3^- : NH_4^+ ratio of 1:3 and 0:4 was 0.041 and 0.54 m moles/day respectively. This is greater than the average daily accumulation of N in the *T. speciosissima* seedlings in these two treatments (0.025 m moles/day) after seed reserves are taken into account. However, the pH data over the 6 weeks of Experiment (b) indicates that the uptake of NH_4^+ was much greater towards the end of the Experiment as the plants grew larger. This supports the conclusion that loss of NH_4^+ from the media was primarily by uptake by the plant rather than by microbial oxidation.

Reduction in the growth of *T. speciosissima* in Experiment 2 (b) when NH_4^+ exceeded more than 75% of the N supply may have been due to N toxicity. As the NH_4^+ content of the irrigation solution increased the leaf N content also increased to high levels when compared to some other members of the family *Proteaceae* (Claassens and Folscher, 1982). The growth of *Banksia serrata* was also optimal when shoot N content was below about 2% (Groves and Keraitis, 1976).

Production of proteoid roots is inhibited by high levels of N in soils or culture solutions (Lamont, 1974). No previous attempt, however, has been made to distinguish between the effect of various N sources on their production. Experiment 2 (b) demonstrated that $NH_4^+$was more effective in inhibiting their production than NO_3^-, despite the greater drop in media concentration of N at high NH_4^+ levels due to uptake of NH_4^+ by the plant. Lamont (1973) demonstrated using a split root zone system technique that it was the external environment, rather than the internal nutrient status of the plant which determined whether or not proteoid roots were produced. Therefore the inhibition of proteoid root formation at high nitrogen levels previously reported may be due to the direct effect of NH_4^+ at the root surface.

Malajczuk and Bowen (1974) and Lamont (1974) demonstrated that proteoid roots do not form under sterile conditions. However neither identified the causal organism (s) nor was there any indication of an invasion of the root by micro-organisms. In both this study and in the work of Moore and Keraitis (1966), proteoid roots were generally formed in large numbers when not inhibited by high levels of nutrients (or in-balances) despite the use of media that was initially sterile (perlite and distilled water). Thus if a specific organism is responsible either it is present on the seed (surface sterilised seed is apparently

free of the micro-organism - Malajczuk and Bowen, 1974) or it is a common airbourne organism, or group of organisms. Proteoid root production has also been associated by a number of authors with high concentrations of organic matter (and hence micro-organisms). However as pointed out previously they are freely produced in perlite which initially has no organic matter. Lamont (1974) demonstrated that a range of micro-organisms, including washings from proteoid roots, were not by themselves responsible for proteoid root production.

Malajczuk and Bowen (1974) suggested that the differences between soils in proteoid root formation may be due to the soil micro-biological status rather than the chemical and physical differences. This experment however, demonstrated that levels of NH_4^+ as low as 0.75 mM could completely inhibit proteoid root production (or lower if NH_4^+ uptake by the plant is considered) whilst higher levels of NO_3^- under the same conditions did not. In most soils NH_4^+ is usually rapidly oxidised by micro-organisms. This study suggests an alternative view to that of Malajczuk and Bowen (1974) i.e. that proteoid root formation is controlled by the soils chemical and physical status which in turn may be affected by its microbiological status.

Lamont (1974) proposed an alternative hypothesis to the formation of proteoid roots being dependant on micro-organisms that was consistent with his observations. It was that a hypothetical inhibitor, which would be required to reach a level that would prevent the formation of proteoid roots but have no effect on non-proteoid roots. This was dismissed by Lamont as being improbable. However the effect of the NO_3^- : NH_4^+ ratio on the proteoid root production was very large compared to its effect on either the total root weight or shoot weight. In many respects NH_4^+ would seem to fit the role of the proposed

theoretical inhibitor. Neither Lamont (1974) or Malajczuk and Bowen (1974) actually measured the level of nutrient availability in the soils they used for determining the effect of micro-organisms on the production of proteoid roots.

CHAPTER 5 THE EFFECT OF N, P AND K FERTILIZERS ON THE GROWTH OF *TELOPEA SPECIOSISSIMA* IN A YELLOW EARTH (EXPERIMENT 3).

Introduction

This experiment examined the effect of N, P and K on the growth of *T. speciosissima* in its native soil. The yellow earth used in this experiment supported a natural population of *T. speciosissima*. The plants were grown in large pots rather than in the field to maximise the number of treatments which could be applied by eliminating the need for buffers.

Materials and Methods

One year old *T. speciosissima* seedlings[1] growing in the field in a yellow earth were transplanted in April 1975, into 30 cm drained pots containing 18 kg (dry weight) of a yellow earth soil. The volume of soil in each pot was approximately 12 litres. One seedling was planted per pot and located in an open field on sheets of corrugated, galvanised iron at the Horticultural Research Station, Gosford and irrigated as required (Plate 5.1).

N, P and K fertilizers were applied in factorial arrangement with 6 replications. The treatments are presented in Table 5.1. There were a total of 36 treatments and the fertilizer applications were split into 26 equal parts and applied weekly. The experiment was terminated 18 months after the seedlings were transplanted (October 1976). Shoots were harvested and dried at 85°C for 48 hours, weighed and then ground in preparation for chemical analysis.

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1. at Mangrove Mountain, N.S.W.

Plate 5.1 General view of Experiment 5.

Table 5.1 Rates of fertilizers added in Experiment 3

Fertilizer	Treatment No.	Total amount added per pot (*m* moles element/pot)
Nitrogen (NH_4NO_3)	N_0	0
	N_1	241
	N_2	482
	N_3	964
Potassium (KCl)	K_0	0
	K_1	43
	K_2	86
Phosphorus (NaH_2PO_4)	P_0	0
	P_1	109
	P_2	218

Chemical analysis of both tissue and soil were done by the Chemistry Branch, Biological and Chemical Research Institute N.S.W. Department of Agriculture, Rydalmere. Total N in both cases was determined after Kjeldahl digestion. Plant P content was determined colorimetrically after digestion of the plant tissue and 'available' phosphorus in the soil by the Bray No. 1 method (Bray and Kurtz, 1945). Soil K and Na were measured by atomic absorption spectrophotometry after extraction with 0.025 N barium chloride. Soil pH was determined on a 1 : 2 (w:v) soil water extract.

Results

(a) Plant growth

(i) Percentage survival

An arcsin $\sqrt{x}$ transformation was done prior to statistical analysis because of the wide range of values (Steel and Torrie, 1960). The only treatment to have a significant ($P < 0.05$) effect on percentage survival was the addition of NH_4NO_3 (Table 5.2). This reduced the survival rate from 81% (65° transformed data) to 6% (14° transformed data) for the highest level of applied N.

(ii) Shoot weight

Treatments N_2 and N_3 (Table 5.1) were eliminated from the statistical analysis of shoot weight because of the high percentage of dead plants.

N, P and K fertilizers all significantly ($P < 0.05$) affected shoot weight. There was also a significant ($P < 0.05$) interaction between N and P (Table 5.3). There were no other significant interactions.

Table 5.2 Effect of added N fertilizer on the percentage survival (arcsin $\sqrt{x}$ transformation) of *T. speciosissima* growing in a yellow earth

	N added per pot (*m* moles)			
	0	241	482	964
% survival (transformed data)	64.5	54.1	24.2	14.0

l.s.d. (P = 0.05) = 13.0

Table 5.3 Effect of N, P and K fertilizer on the shoot dry weight (g) of *T. speciosissima* grown in a yellow earth

(a)	N and P addition		
N added (*m* moles/pot)	P added (*m* moles)		
	0	43	86
0	3.66	11.68	11.73
241	1.79	12.19	25.78

l.s.d. (P = 0.05) = 5.73

(b)	K addition		
	K added per pot (*m* moles)		
	0	109	218
shoot weight (g)	23.78	19.76	14.03

l.s.d. (P = 0.05) = 2.86

Shoot weight generally increased with increasing N and P. The N x P interaction occurred, however, because no significant increase ($P > 0.05$) in shoot weight occurred at N_0 when P was increased from P_1 to P_2 whilst at N_1 a significant ($P < 0.05$) increase occurred. Increasing the level of K decreased shoot weight.

(b) Tissue analysis

Due to the high percentage of deaths in treatments N_2 and N_3 and the resultant limited quantity of plant material these treatments were not included in the statistical analysis of tissue nutrient levels.

(i) N (Table 5.4)

There was a significant interaction ($P < 0.05$) between N and P; addition of N increased the tissue N level only when P was added. K had no significant ($P > 0.05$) effect on shoot N. The % N in the surviving plants of treatments N_2 and N_3 was 1.33 (s.e. = 0.27) and 1.23 (s.e. = 0.19) respectively.

(ii) P (Table 5.5)

Addition of P increased tissue P levels. There was also significant interaction ($P < 0.05$) between N and P; when no P was added additional N caused a decrease in the leaf P level. When 218 m moles P/pot was added additional N significantly ($P < 0.05$) increased tissue P.

(iii) K

Addition of N and K fertilizers had a significant ($P < 0.05$) effect on leaf tissue levels of K. No interactions were significant ($P > 0.05$). Increasing the level of K fertilizer significantly ($P < 0.05$) increased the shoot level of K (Table 5.6). Increasing the N level from 0 to 241 m moles/pot (N_0 to N_1) significantly ($P < 0.05$) decreased the tissue level of K from 1.00% to 0.74%. P fertilizer had

Table 5.4 Effect of N and P on the percentage of N in the shoot of *T. speciosissima* grown in a yellow earth

Amount of N added per pot (*m* moles)	Amount of P added per pot (*m* moles)		
	0	109	218
0	1.17	1.03	1.05
241	1.21	1.24	1.46

l.s.d. (P = 0.05) = 0.19

Table 5.5 Effect of N and P on the percentage P in the tissue of *T. speciosissima* grown in a yellow earth

Amount of N added per pot (*m* moles)	Amount of P added per pot (*m* moles)		
	0	109	218
0	0.050	0.062	0.076
241	0.033	0.064	0.101

l.s.d. (P = 0.05) = 0.016

Table 5.6 Effect of K fertilizer application on the percentage K in the shoot of *T. speciosissima* grown in a yellow earth

	Amount of K added per pot (*m* moles)		
	0	43	86
% K in shoot	0.479	0.963	1.173

l.s.d. (P = 0.05) = 0.122

no significant effect ($P > 0.05$) on shoot K levels.

(iv) Na (Table 5.7)

Only increasing the level of P fertilizer significantly ($P < 0.05$) affected shoot Na percentage. There was a small increase in the shoot Na percentage as the level of P fertilizer was increased.

(c) Nutrient status of the soil

One bulked sample of each treatment was taken at the conclusion of the experiment. Statistical analysis was as a 3 factor factorial. The third level interaction was used as an estimate of variance.

(i) Total N

Addition of N, P or K fertilizers had no significant effect ($P > 0.05$) on the total N level of the soil nor were any interactions significant. The mean N level was .046% (s.e. = 0.001)

(ii) P (Table 5.8)

Due to the wide range of P values,a log (x + 1) transformation was applied to the data before statistical analysis (Steel and Torrie, 1960). Addition of P significantly increased ($P < 0.05$) the level of available P in the soil. The N x P interaction was also significant ($P < 0.05$). When no P was added, increasing the level of N significantly increased ($P < 0.05$) the level of available P by a small amount. This effect was not found when P fertilizer had been added. Addition of K fertilizer did not significantly ($P > 0.05$) effect soil P levels.

(iii) K (Table 5.9)

Addition of N or K had a significant effect ($P < 0.05$) on the level of available K in the soil. The interaction was also

Table 5.7 Effect of P fertilizer on the percentage Na in the shoot of *T. speciosissima* grown in a yellow earth

	Amount of P added per pot (*m* moles)		
	0	109	218
% Na in shoot	0.040	0.045	0.051

l.s.d. (P = 0.05) = 0.008

Table 5.8 Effect of fertilizer addition on the available P content(log (x + 1) transformation) of a yellow earth (ppm).

Amount of N added per pot (*m* moles)	Amount of P added per pot (*m* moles)		
	0	109	218
0	0.59	1.91	2.13
241	0.71	1.83	2.13
482	0.80	1.75	2.03
964	0.89	1.81	2.08

l.s.d. (P = 0.05) = 0.16

Table 5.9 Effect of fertilizer addition on the exchangeable K content (ppm) of a yellow earth

Amount of N added per pot (*m* moles)	Amount of K added per pot (*m* moles)		
	0	43	86
0	39	181	312
241	23	105	157
482	16	60	130
964	12	59	103

l.s.d. (P = 0.05) = 58

significant ($P < 0.05$). The effect of added P or any other interaction was not significant ($P > 0.05$). As the level of K fertilizer increased, the exchangeable K also increased significantly ($P < 0.05$) and as the level of N fertilizer increased the level of exchangeable K fell (where K was added). However, there was no significant ($P < 0.05$) change in the soil K level as N increased when no K was added.

(iv)

All the fertilizers had a significant effect ($P < 0.05$)) on pH, however there were no significant ($P > 0.05$) interactions. Addition of NH_4NC_3 caused a reduction in pH with the highest rate resulting in a 1.05 pH reduction. The highest rate of KCl and NaH_2PO_4 resulted in increases of 0.52 and 0.22 pH units respectively (Table 5.10).

Discussion

Increasing the level of applied N especially above 241 m moles/pot resulted in high mortality. Similar results have been noted by other workers where applied N has adversely affected the establishment of heath plants including members of the family *Proteaceae*(Heddle and Specht, 1975; Groves and Keraitis, 1976).

Direct N toxicity is unlikely to have caused the deaths in this experiment.

Goodwin (1982) found that 857 m moles N per 12 litres (Experiment 2 of his experiments) as urea formaldehyde - over 67% of which would be available in 1 year (Bunt, 1976), was not toxic to *T. speciosissima*. It has also been reported that *T. speciosissima* is tolerant to "high" levels of mixed inorganic fertilizers (Anon, 1972).

Table 5.10 Effect of fertilizer addition of the pH of a yellow earth

(a) N fertilizer addition

	Amount of N added per pot (*m* moles)			
	0	241	482	964
pH	5.54	4.81	4.59	4.49

l.s.d. (P = 0.05) = 0.17

(b) P fertilizer addition

	Amount of P added per pot (*m* moles)		
	0	109	218
pH	4.77	4.83	4.98

l.s.d. (P = 0.05) = 0.15

(c) K fertilizer addition

	Amount of K added per pot (*m* moles)		
	0	43	86
pH	4.68	4.80	5.10

l.s.d. (P = 0.05) = 0.15

The very low soil N levels at the conclusion of the experiment, even for the high N treatments, showed that there was no significant accumulation of N compounds in the soil. Uptake by the plants was not sufficient to account for this. The applied NH_4NO_3 was thus either leached or biologically destroyed during the course of the experiment.

Tissue N levels in the surviving plants even at the highest level of applied N were well below those causing death in a wide range of *Proteaceae* which also grow naturally in soils deficient in nutrients for the normal growth of most crop plants (Claassens and Folscher, 1982). The tissue level of N was also approximately half that at which maximum growth occurred in Experiment 2.

Addition of N in this experiment reduced soil pH. The severity of the effect of *Phytophthora* on the root system of avocado is influenced by soil pH (Baker, 1978). However, it is unlikely that this reduction was responsible for the mortalities that occurred because addition of P and K fertilizers in this experiment caused similar (but opposite) changes in soil pH to that caused by NH_4NO_3 levels, but had no effect on the mortality rate. Addition of NH_4NO_3 thus may have directly increased susceptibility of *T. speciosissima* to *P. cinnamomi* or some other similar disease-causing organism. *T. speciosissima* is attacked by *P. cinnamomi* and other "water moulds" known to be endemic in the soil used in this experiment (P. Barkley, personal communication).

Increasing the rate of applied P increased shoot weight, tissue P levels and soil P levels while not causing any visible toxicity. Although the soil type used in this experiment is known to

fix P, the final level of available P at the highest rate applied was still 3 to 4 times the minimum for the growth of exotic pasture species (Wright *et al.*, 1975). Thus, unlike many members of the *Proteaceae* for which relatively low levels of P in the growing medium (compared to that required by many other species for adequate growth) may cause a P toxicity, *T. speciosissima* exhibited no such toxicity. It also responded, in terms of its growth rate, to high levels of added P. The tissue level of P at which the greatest growth rate occurred in this experiment was about 0.1%. This was at the highest level of added P in this experiment. In Experiment 2 the tissue level of P was approximately 3 times higher, yet there were no visual signs of P toxicity and the growth of plants was adequate in that experiment. This suggests that the highest level of P added in this experiment was actually sub-optimal for growth of the seedlings.

The highest level of added P in this experiment was at least 40% greater on a volume basis than that reported by Goodwin (1982) to cause a P toxicity in *T. speciosissima*. However, in his experiment in which he examined the effect of P on *T. speciosissima*, the plants grew well at the highest rate of applied P for the first 4 months. This rate is at least twice that recommended by Handreck and Black (1984) for growing plants not sensitive to P.

The basis for Goodwin's claim that *T. speciosissima* is sensitive to P appears to be that 2 out of a total of 3 plants in his highest P treatment died after the initial 4 month period. The third plant apparently exhibited no signs of P toxicity. However, after the initial period the plants were subjected to various forms

of stress (frost, heat and water stress) and the deaths were probably due to these factors. No supporting statistical analysis was given.

Sodium added in the P fertilizer, had no significant effect on soil Na levels and little effect on tissue Na levels, presumably because it is readily leached from the soil. Na^+ is the cation least strongly-held by the clay fraction compared with NH_4^+, K^+, Mg^{2+} and Ca^{2+} ions (Bunt, 1976). It also had little effect on plants in Experiment 2. Thus the Na associated with the P fertilizer is unlikely to have had any significant effect on the growth of the *T. speciosissima* seedlings in this experiment.

Addition of K increased the level of exchangeable K in the soil while addition of N (as NH_4NO_3) decreased it, possibly by NH_4^+ ions replacing K ions in the soil exchange complex thus making them more susceptible to leaching. However, only addition of K had a significant effect on the tissue levels of K.

Despite the soil being initially deficient in K for the growth of many exotic crops (Wright *et al.*, 1975) added K reduced the dry weight of the shoots. Compared with a wide range of exotic species, the amount of K in leaf tissue would indicate that *T. speciosissima* was deficient in K (Bunt, 1976; Meynhardt, 1976), but many proteas, with comparatively very low levels of tissue K ($\sim$ 0.18%), do not respond to added K (Meynhardt, 1976). Thus either there is sufficient K in the soil for normal growth of *T. speciosissima* despite the low tissue level, or the optimal level of K fertilizer is below 43 m moles K per 18 kg soil added over a one year period. Experience in South Africa with *Protea* on soils of low natural

fertility where there was no response to added K fertilizers, suggests that the former is the case (Meynhardt, 1976). The relatively high level of K in the shoots in Experiment 2 (b) suggests that the basal level of K used in that experiment may have been supraoptimal.

The Cl associated with the K fertilizer may have been responsible for the reduction in the shoot weight. However, this is unlikely as Cl, like Na, would have been readily leached from the pots (Bunt, 1976) and added Cl had little effect on the growth of *T. speciosissima* seedlings in Experiment 2.

CHAPTER 6 THE EFFECT OF N, P, K AND *PHYTHPHTHORA CINNAMOMI* ON THE GROWTH OF *TELOPEA SPECIOSISSIMA* IN PERLITE (EXPERIMENT 4).

Introduction

Results of Experiment 3 showed that adding NH_4NO_3 to a soil caused a high mortality of *T. speciosissima* seedlings. However in Experiment 2 (a) a high level of NH_4NO_3 in the sterile perlite medium did not cause any mortalities, despite high tissue levels of N.

This supports the conclusion made in Experiment 3 that the deaths were not directly due to NH_4NO_3, but possibly *Phytophthora cinnamomi*.

The aim of this experiment is to examine the effects of nutrients (N, P and K) and *P. cinnamomi* on the growth and survival of *T. speciosissima* seedlings in an initially sterile medium.

Materials and Methods

Seedlings of *T. speciosissima* were grown as in Experiment 2 (a). The basal nutrient solution however, contained 4.0 mM Ca^{2+}, 2.0 mM Mg^{2+}, 6.0 mM SO_4^{2-} and trace elements at the rates used in Hoagland's solution (Arnon, 1938). As in Experiment 2 (a) each pot (containing 2 *T. speciosissima* seedlings) received a total of 1 litre of the basal solution in 20 equal applications of 50 ml each, 3 times a week, beginning when the seedlings were thinned.

The amounts of additional nutrients added per pot (treatments) are given in Table 6.1. Variation in pH was minimised by using NH_4NO_3 as the N source. The concentrations of Na^+ and Cl^- were minimised by the selection of appropriate sources of P and K. Half the additional nutrients were applied at thinning and the other half one week later.

Table 6.1 Fertilizer treatments used in the Experiment - The effect of N, P, K, and *P. cinnamomi* on the growth of *T. speciosissima* (Experiment 4). Fertilizers were applied in a factorial arrangement.

Fertilizer	Amounts of nutrients added per pot (m moles/element) Treatment No.				
	0	1	2	3	4
N (as NH_4NO_3)	0	3.6	7.2	14.4	28.8
P (as KH_2PO_4 and Na H_2PO_4)	0	3.2	6.4	9.8	-
K (as KH_2PO_4 and KCl)	0	1.3	2.6	5.2	-

Half the pots were inoculated two weeks after thinning with *P. cinnamomi* isolated from a diseased plant of *T. speciosissima* by burying 1 cm below the surface, two, 2 cm long pieces of autoclaved lucerne stem that had been inoculated 15 days previously with *P.cinnamomi*. Uninoculated pieces were used for the remaining pots.

All possible combinations of the above treatments were applied giving a total of 160 treatments (5 N levels, 4 P levels, 4 K levels, + and - *P. cinnamomi)*. The pots were randomised and there was no replication.

The dry weights of the shoots, proteoid roots and non-proteoid roots were determined at the termination of the experiment (10 weeks after thinning) and the roots were visually rated in accordance with Table 6.2. The results were analysed as a four factor factorial with two sub-samples but no replication. The fourth order interaction and the two sub-samples were used as an estimate of variance.

Results

(a) Effects on the Root System

(i) Damage score

All the main effects and the following interactions were significant ($P < 0.05$), N x P, N x K and *P. cinnamomi* x N interactions.

N x *P. cinnamomi* interaction (Figure 6.1).

Increasing levels of N in the absence of *P. cinnamomi* had no effect on the damage score, nor did it in the presence of *P. cinnamomi* except at the highest level of N

N x P interaction (Figure 6.2).

As the level of N increased, increasing levels of P decreased the damage score except at the highest level of N where increasing levels

Table 6.2 Ratings used to score root rot caused by *P. cinnamomi*

Root rot rating	Percentage of non-proteoid roots with dead tips
0	up to 5
1	5 to 50
2	51 to 95
3	over 95

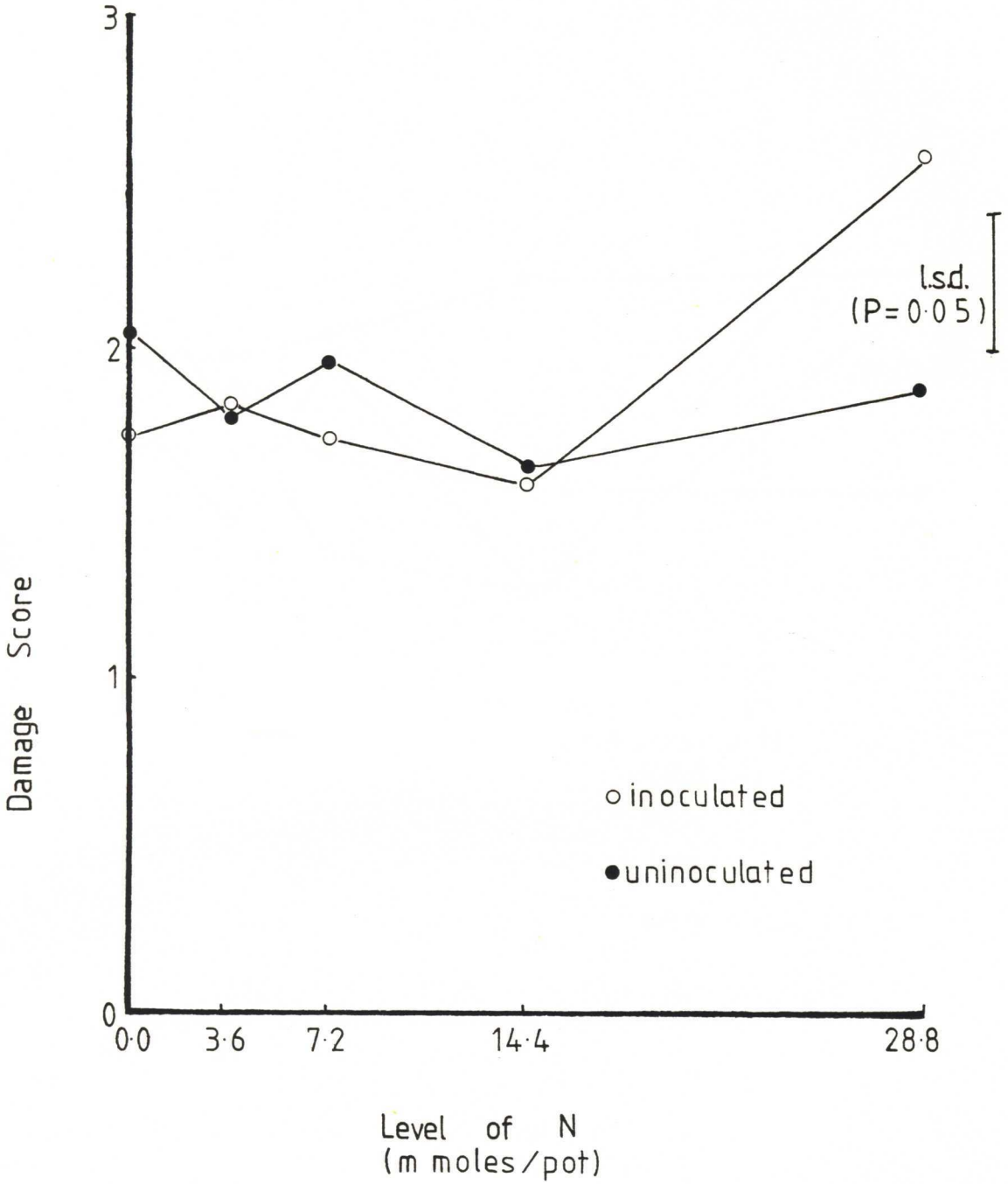

Figure 6.1. Effect of inoculation with *Phytophthora cinnamomi* and level of N fertilizer on the damage score of *T. speciosissima* roots.

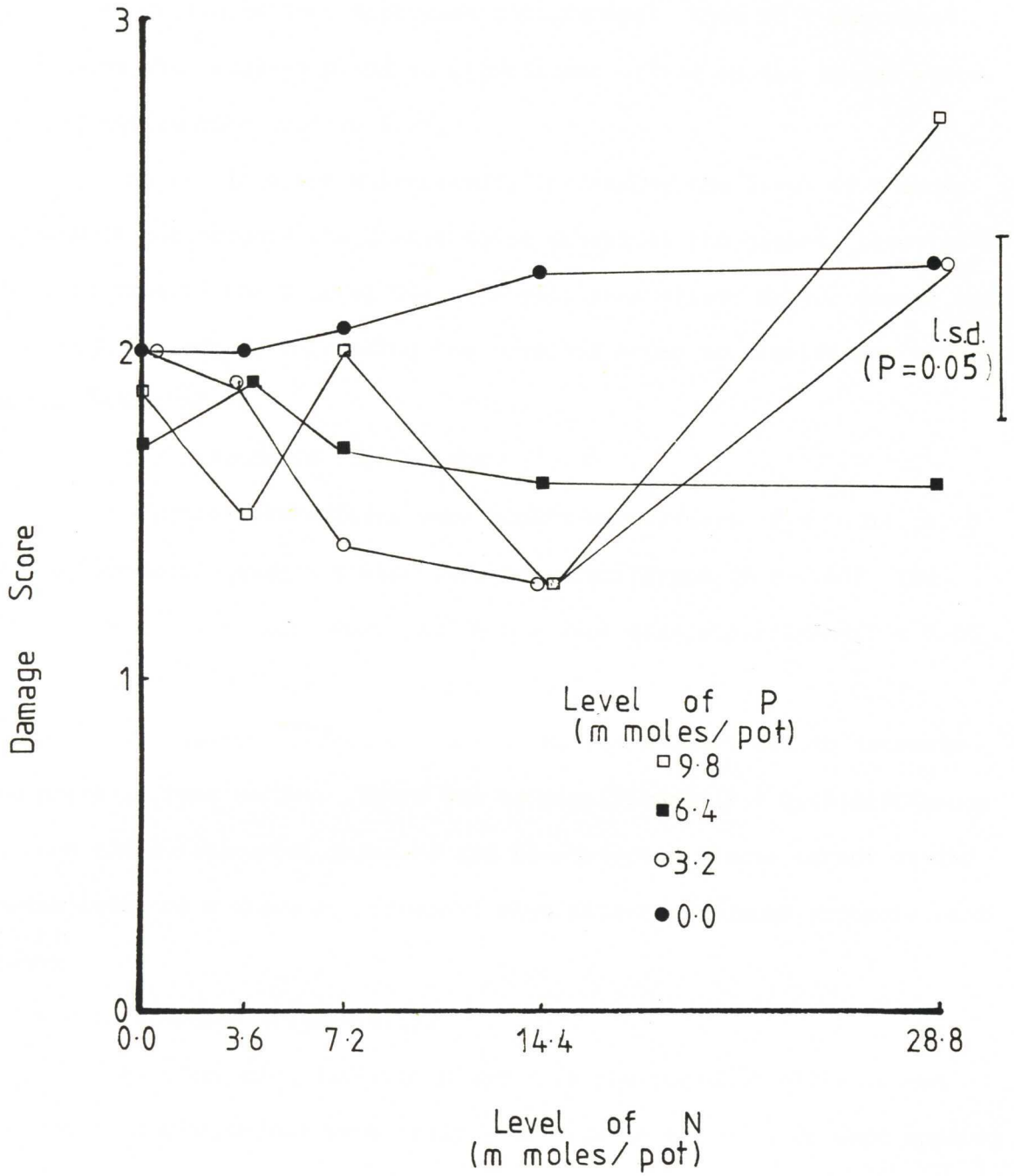

Figure 6.2. Effect of the level of N and P fertilizers on the damage score of the roots of *T. speciosissima*.

of P first decreased then increased root damage. When no P was added, increasing the level of N had no significant effect on the damage score.

N x K interaction (Figure 6.3).

As the level of N increased, increasing the level of K above 1.3 m mole/pot reduced the damage score except at the highest level of N, where increasing the K level had no significant effect on the damage score. When no K was added, increasing the level of N had no significant effect on the damage score.

(ii) Proteoid Root Weight

All the main effects were highly significant ($P < 0.01$) (N, P and *P. cinnamomi*),except K which was not significant ($P > 0.05$). The interactions N x *P. cinnamomi* and N x P were also significant($P < 0.05$).

P. cinnamomi x N interaction (Figure 6.4).

The general effect of increasing the N level was to decrease the proteoid root weight. There was no significant ($P > 0.05$)difference between the *P. cinnamomi* infected and non-infected plants except at the lowest level of N where *P. cinnamomi* significantly reduced proteoid root weight.

N x P interaction (Figure 6.5).

An increasing level of N and P in the pot generally reduced the proteoid root weight especially when high levels of both were applied together. The general increase in the proteoid root weight when 3.6 m moles of N per pot was added was due to the effect of the interaction between *P. cinnamomi* and N.

(iii) Non-proteoid Root Weight

The effect of added N, P, K and *P. cinnamomi* on non-proteoid root weight was highly significant ($P < 0.01$). The N x *P. cinnamomi and*

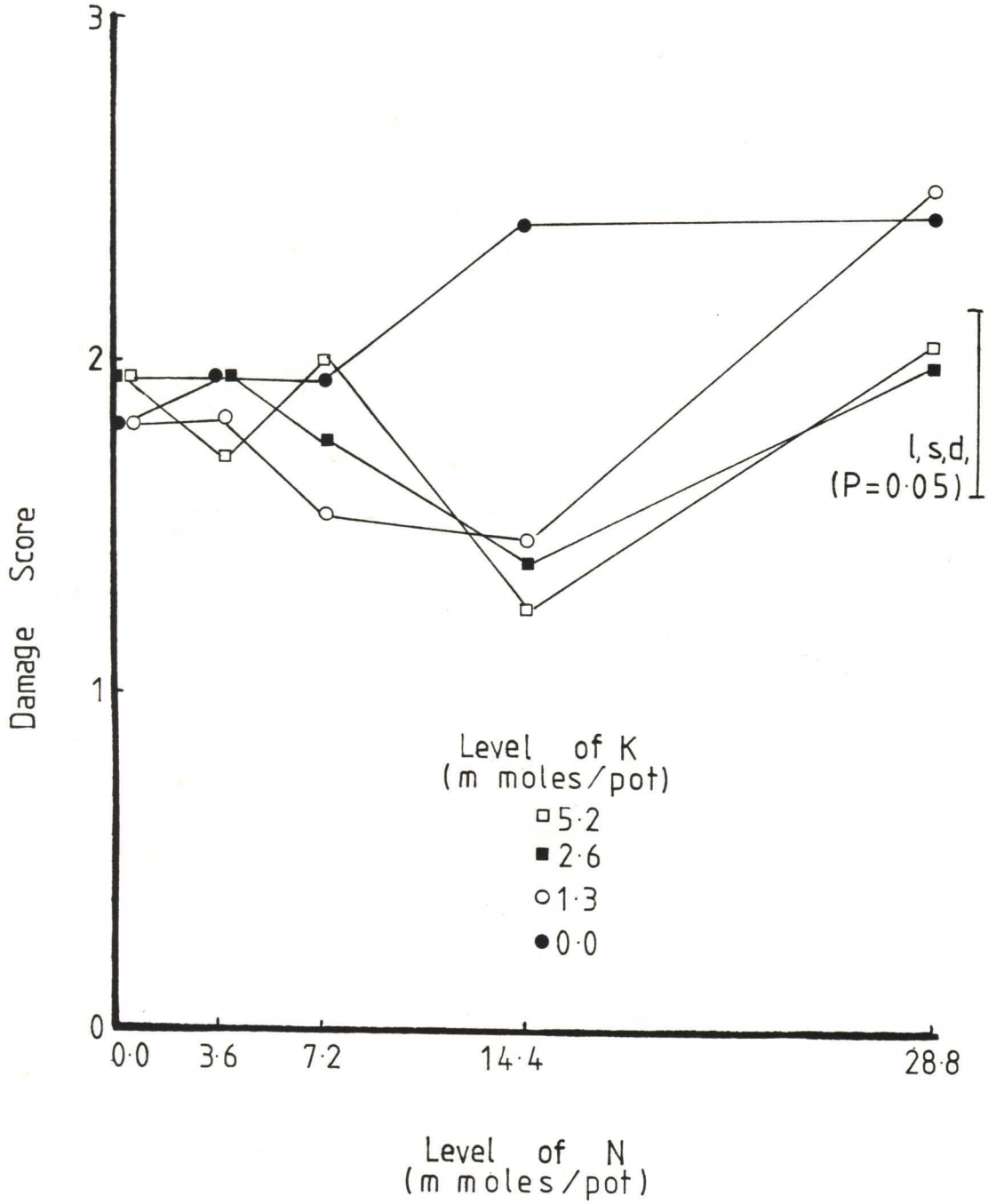

Figure 6.3. Effect of N and K fertilizers on the damage score of *T. speciosissima* roots.

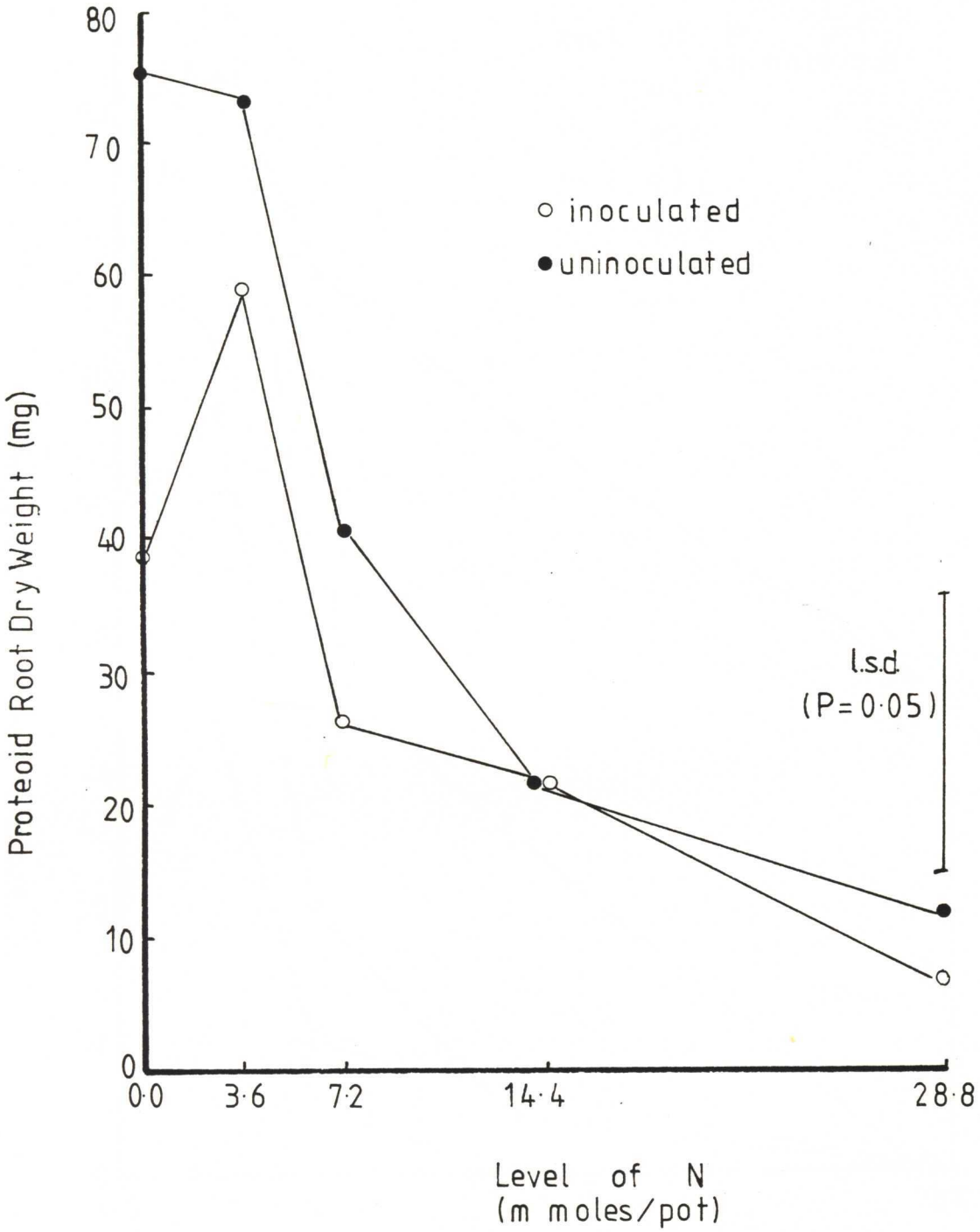

Figure 6.4 Effect of inoculation with *Phytophthora cinnamomi* and level of N fertilizer on the proteoid root dry weight of *T. speciosissima*.

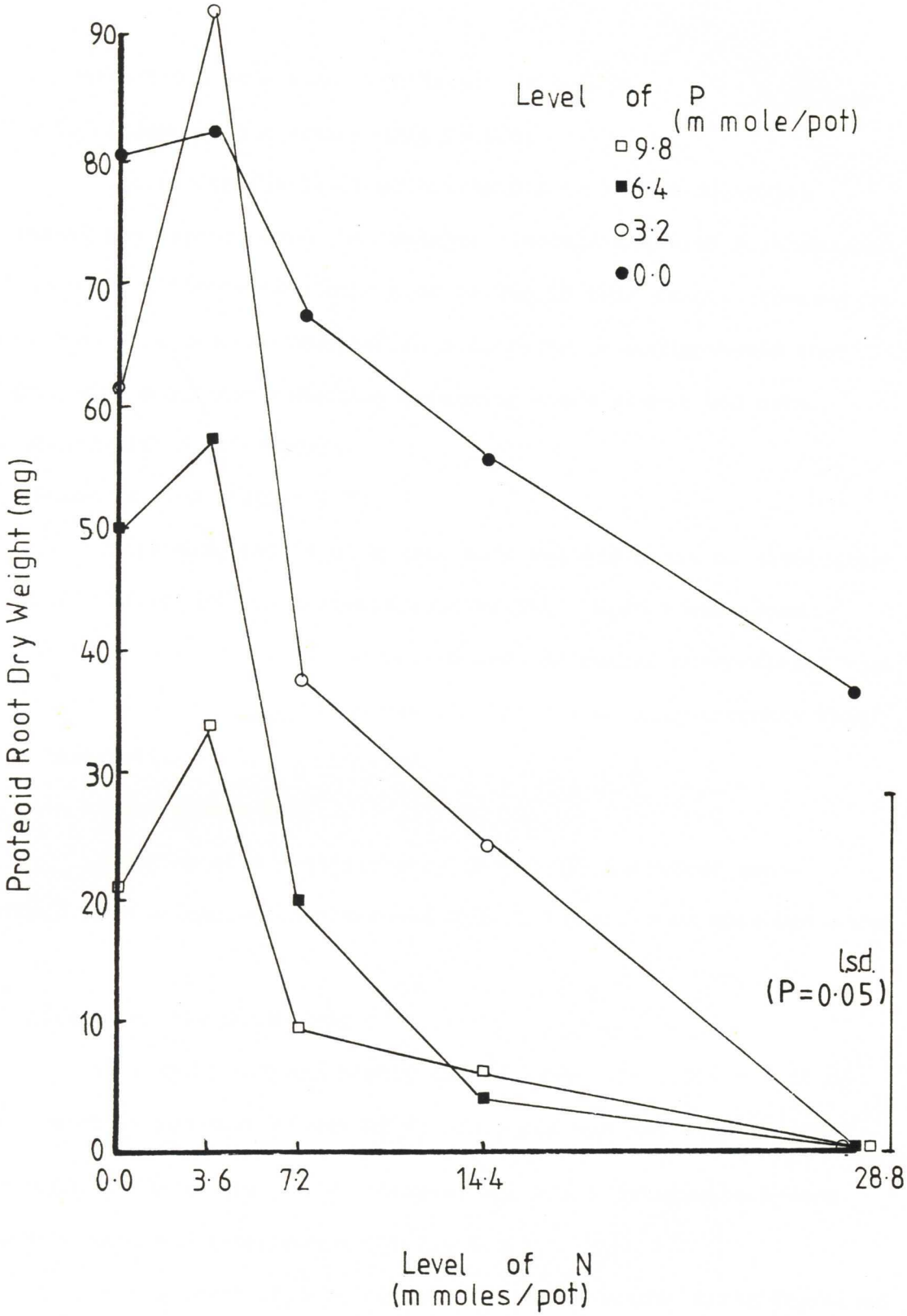

Figure 6.5 Effect of level of N and P fertilizers on the proteoid root dry weight of *T. speciosissima*.

N x P interactions were also significant ($P < 0.05$)

N x *P. cinnamomi* interaction (Figure 6.6).

Increasing the level of N from 0.0 to 14.4 m moles/pot increased the non-proteoid root weight. Inoculation with *P. cinnamomi* did not effect the non-proteoid root weight in this range. However an additional 14.4 m moles resulted in a decrease in non-proteoid root weight, with a greater reduction occurring where plants had been inoculated with *P. cinnamomi*.

N x P interaction (Figure 6.7)

Increasing levels of N when no P was added had no significant ($P > 0.05$) effect on non-proteoid root weight. When P was added increasing levels of N first increased then decreased non-proteoid root weight with 5.4 and 9.8 m moles/pot eliciting a greater response than 3.2 m moles/pot.

K main effect (Table 6.3).

Addition of K significantly ($P < 0.05$) increased non-proteoid root weight which plateaued when 1.3 m moles or more was added per pot.

(b) Effects on the shoot weight.

N, P and K all had highly significant ($P < 0.01$) effect on shoot weight. The main effect of *P. cinnamomi* was not significant ($P > 0.05$) although the N x *P cinnamomi* and N x P interactions were.

P. cinnamomi x N interaction (Figure 6.8.)

As the level of N increased, the shoot weight first increased then decreased. There was no significant difference between inoculated and uninoculated plants except at the highest level of N, where the inoculated plants had a significantly lower shoot weight.

N x P interaction (Figure 6.9).

When no P was added, increasing levels of N had no effect on shoot weight. However, when P was added, increasing the level of N first

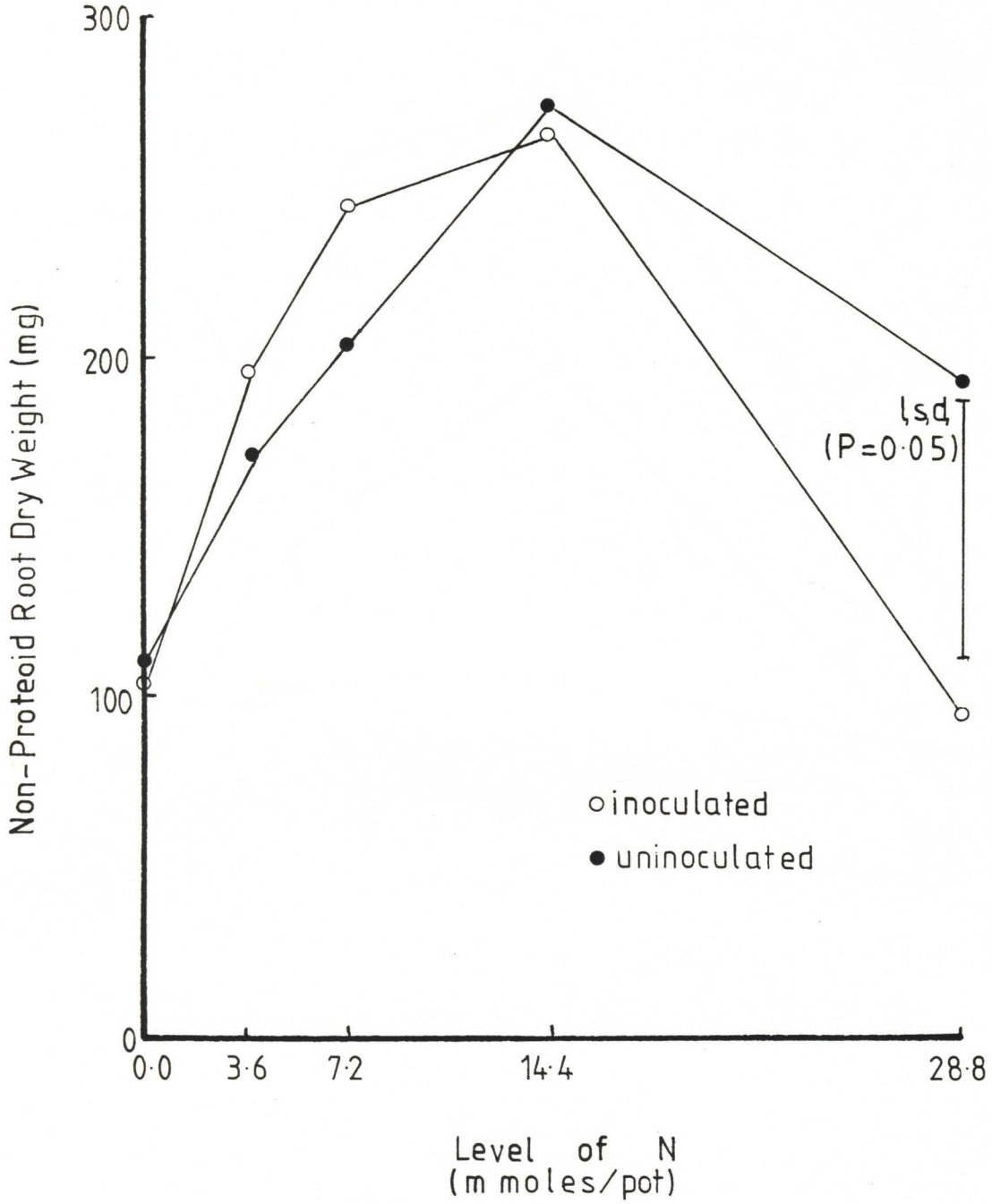

Figure 6.6 Effect of inoculation with *Phytophthora cinnamomi* and level of N fertilizer of the non-proteoid root dry weight of *T. speciosissima*.

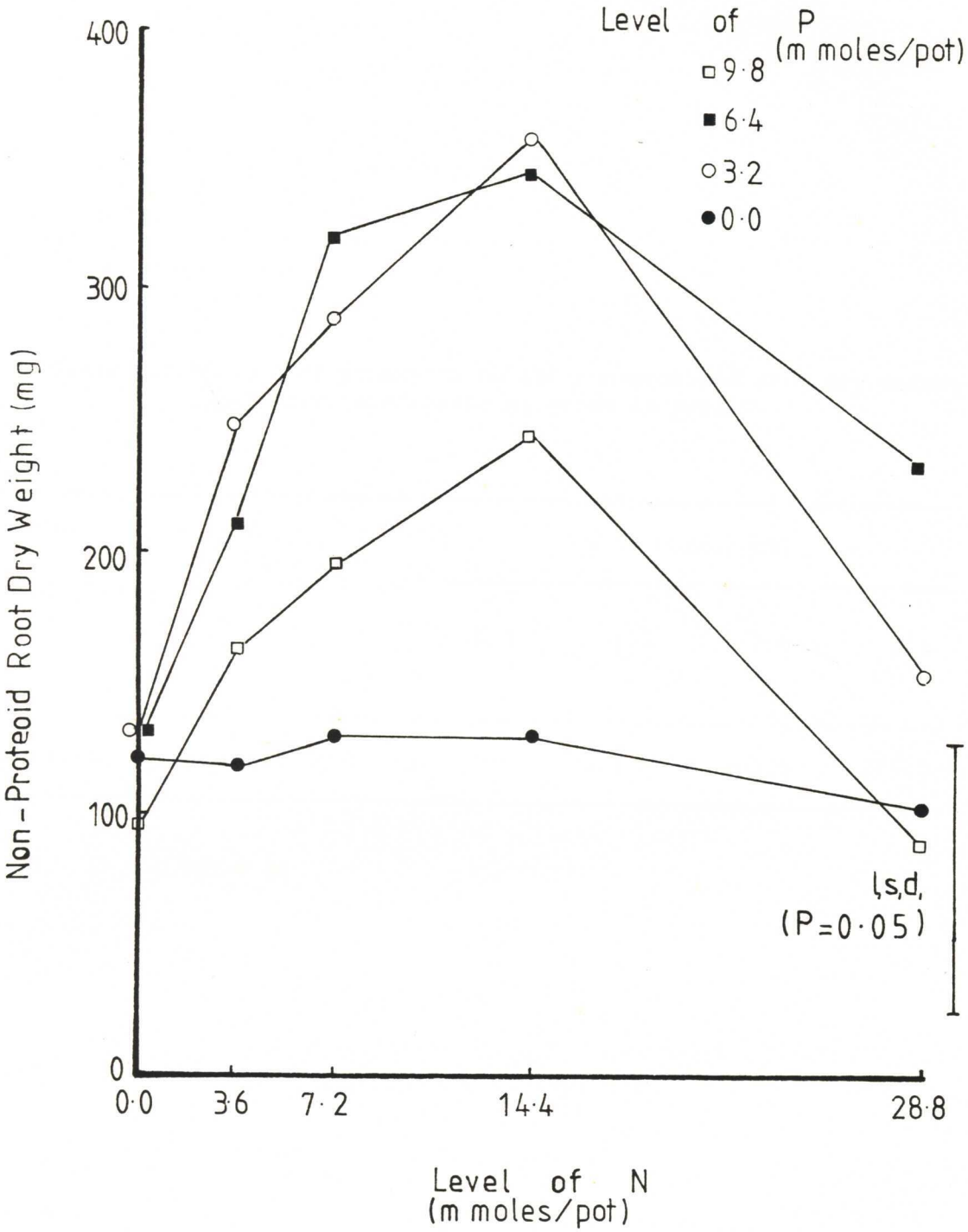

Figure 6.7 Effect of N and P fertilizers on the non-proteoid root dry weight of *T. speciosissima*.

Table 6.3 Effect of potassium on the non-proteoid root dry weight (mg) of *T. speciosissima* grown in perlite.

	K (m moles/pot)			
	0.0	1.3	2.6	5.2
Non-proteoid root dry weight (mg)	149.0	201.0	209.0	205.0

l.s.d. (P = 0.05) = 48

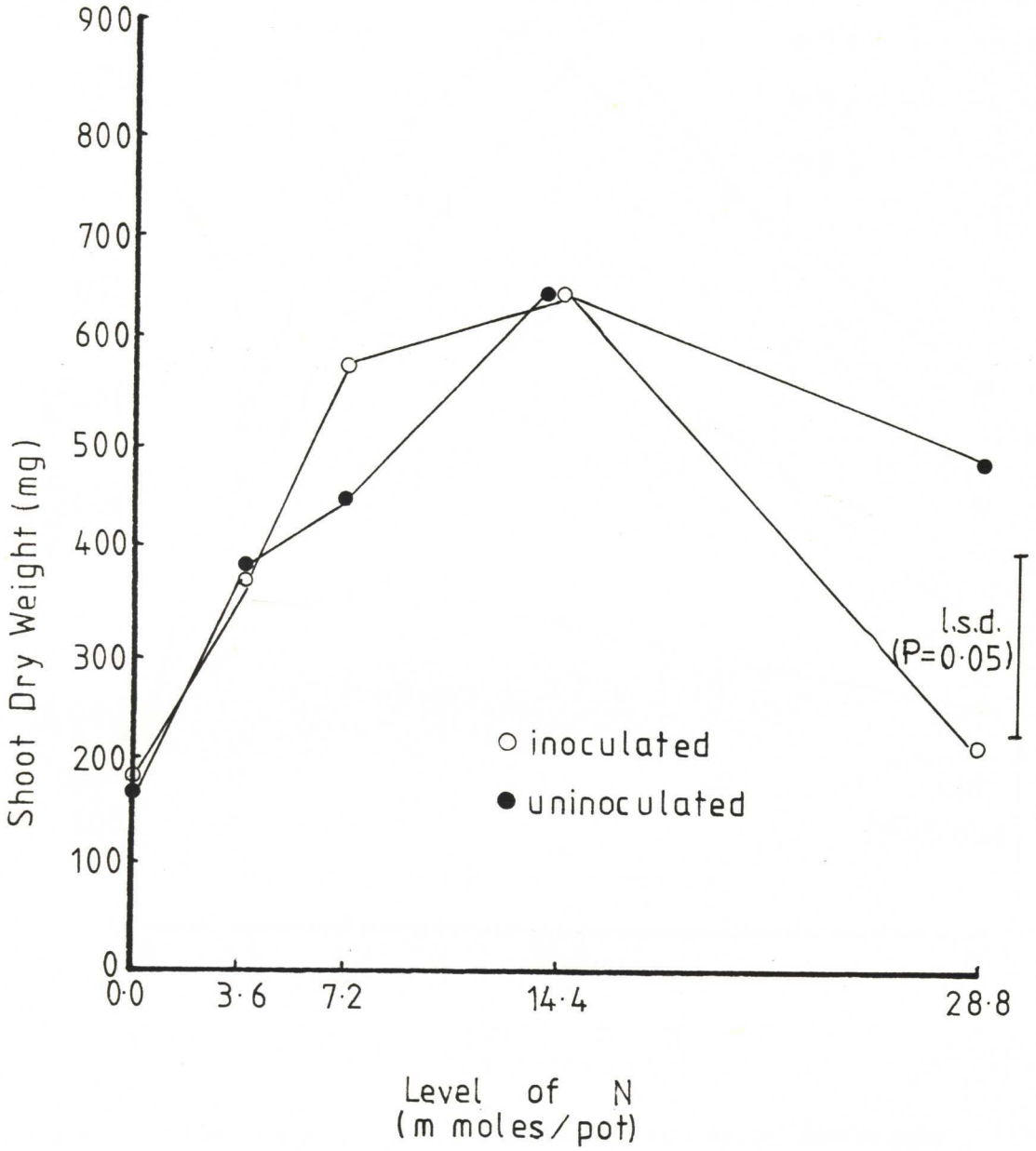

Figure 6.8 Effect of inoculation with *Phytophthora cinnamomi* and level of N fertilizer of the shoot dry weight of *T. speciosissima*.

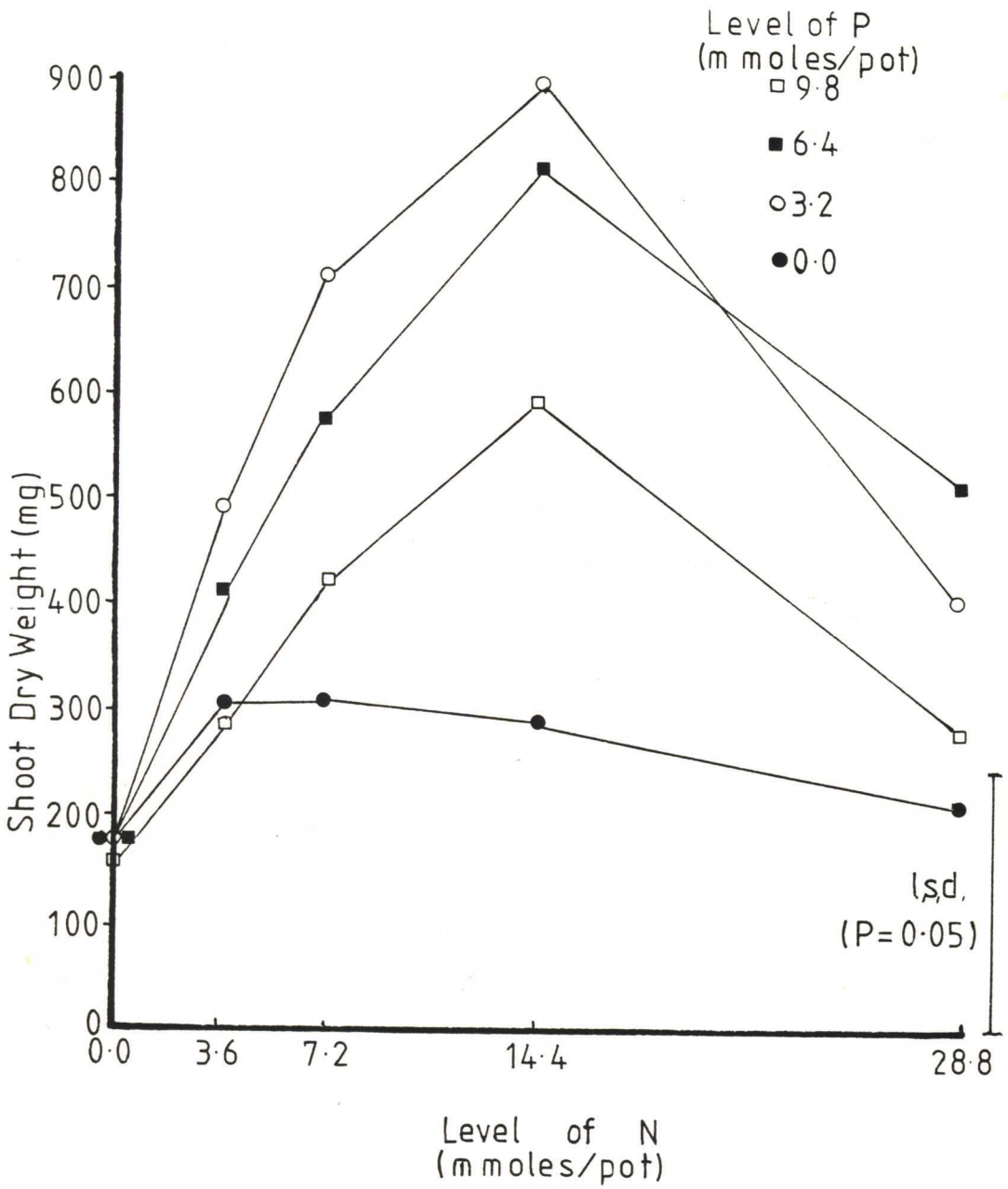

Figure 6.9 Effect of N and P fertilizers on the shoot dry weight of *T. speciosissima*.

increased shoot weight and then decreased it. This effect was greatest at the 2 highest levels of added P.

K main effect (Table 6.4).

Addition of K up to 1.3 (m moles/pot)increased shoot weight, however above this level there was no significant ($P > 0.05$) increase.

Discussion

Phytophthora cinnamomi caused a reduction in the shoot and non-proteoid root weights and caused damage to the non-proteoid roots only at the highest level of N. This supports the conclusion that the presence of *P. cinnamomi* in the soil in Experiment 3 may have been responsible for the deaths of the *T. speciosissima* seedlings at high levels of applied N (Table 6.5). The severity of the effect of *P. cinnamomi* depends on soil aeration (Baker, 1957) with a greater severity in poorly drained soils. The yellow earth used in Experiment 3 was poorly aerated compared to the perlite used in this Experiment.

Added N was necessary for the plant to respond to added P. There was probably enough available N in the yellow earth used in Experiment 3 for the *T. speciosissima* to respond to added P when no N was added. There was little evidence of phytotoxicity to P except for a reduction in growth at the highest level applied and then only at the two highest levels of N (besides the effects on proteoid roots)in this Experiment. This supports the conclusion reached in Experiment 3 that *T. speciosissima* is not particularly sensitive to P.

Increasing the level of K from 0.0 to 1.3 m moles/pot increased proteoid and non-proteoid root weight and shoot weight but further increases were not significant. There was no evidence of phytotoxicity to K even at the highest rate applied. Thus, although the K rate used

Table 6.4 Effect of K on shoot dry weight (g) of *T. speciosissima* grown in perlite.

	K (m moles/pot)			
	0.0	1.3	2.6	5.2
Shoot dry weight (mg)	590.0	840.0	900.0	940.0

l.s.d. (P = 0.05) = 220

Table 6.5 . Summary of the effects of N, P and K fertilizers and *P.cinnamomi* on the growth of *T. speciosissima* in Experiments 2, 3 and 4.

Factor	Plant response		
	Shoot growth	Root growth	
		Non-proteoid	Proteoid
1. Nitrogen			
(a) In perlite	Growth increases then decreases with increasing N. NH_4^+ more readily taken up by the plant than NO_3^-. Added P is necessary before the plant can respond to added N.	Similar to shoot growth.	Growth is generally inhibited by N, especially NH_4^+ or in combination with high P levels.
(b) In a yellow earth	Increased growth occurred with added N only when P was also added. High levels of added N resulted in plant deaths which were apparently not related to N uptake.	-	-
2. Phosphorus			
(a) In perlite	Growth generally increased with increasing P level. The response to P depends on the N and to a lesser extent the K level in the medium.	Similar to shoot growth but no K interaction.	Increasing levels of P, especially in combination with high levels of N inhibit growth.
(b) In a yellow earth	Best growth occurred at the highest level of added P especially when N was also added.	-	-
3. Potassium			
(a) In perlite	Plant has a low K requirement.	Similar to shoot growth.	No effect.
(b) In a yellow earth	Added K reduced shoot growth.	-	-
4. *Phytophthora cinnamomi*			
(a) In perlite	Reduced shoot growth only at high levels of added N.	Similar to shoot growth.	Reduced proteoid root weight only at low (zero) N levels.
(b) In a yellow earth	May have caused plant death at high levels of added N.	-	-

in Experiment 2 (a) was above that needed for optimum growth, it probably had no deliterious effect on the growth of *T. speciosissima* in that Experiment. As was found for *T. speciosissima* growing in the yellow earth in Experiment 3, *T. speciosissima* has a low K requirement compared to exotic plants such as pasture grasses (Wright *et al.*, 1975), possibly reflecting the low level of K in the soils in which it has evolved.

Phytophthora cinnamomi affected the proteoid and non-proteoid systems at different levels of applied N. The weight of the proteoid root system was only reduced by *P. cinnamomi* at low levels of N. Higher N levels 'protected' proteoid roots against infection, however high N levels also inhibited their growth. The mechanism of resistance to infection may be one of chemical damage to the roots by the N compounds, producing phenols and associated compounds which inhibit invasion by *P. cinnamomi*. Gilpatric (1969) found that ammonia damage to avocado roots made them less susceptible to invasion by *P. cinnamomi*, while Zentmyer *et al.* (1955) suggested that nitrite damage to avocado roots may retard the development of the disease.

Loss of the proteoid roots at the zero N level probably did not affect the top weight in this Experiment because the only source of N would have been plant reserves. However, in their natural environment the loss would have been more critical to the growth of the plant at low levels of N because of the intense exploration offered by the roots, especially in relation to extracting small amounts of N and other nutrients from decaying organic matter. The loss of roots to *P. cinnamomi* would also be more critical if severe moisture stress

occurred. However in cultivation the role of proteoid roots will be greatly reduced because levels of applied N and P which are optimal for vegetative growth will inhibit their development.

Lamont (1972b) described the relationship between proteoid and non-proteoid roots as going through four phases as nutrient availability increases (see the Review of Literature for more details). He claimed that this relationship held true for increasing levels of N and P as well as organic matter.

This relationship held true for *T. speciosissima* in this Experiment but only when infected with *P. cinnamomi*. In the absence of *P. cinnamomi* the first phase i.e. an increase in proteoid root production as non-proteoid root growth increases was absent. The other 3 phases were as described by Lamont, whether or not the *T. speciosissima* had been inoculated with *P. cinnamomi*.

Another possible reason for the existence of the first phase in Lamont's experiment is that his *Hakea* plants were infected with *P. cinnamomi*, or a disease-causing organism with a similar effect, since the organic matter used in his experiments had not been sterilized. Lamont's experiments with *Hakea* and the present work using *T. speciosissima* were carried out utilizing similar nutrient concentrations and the results for *Hakea* plants were similar to those for inoculated *T. speciosissima* plants.

PART III

CHAPTER 7 GROWTH OF SELECTED CLONAL MATERIAL OF *T. SPECIOSISSIMA* IN THE FIELD (EXPERIMENT 5)

Introduction

Telopea speciosissima has proved to be unreliable in cultivation (Brackpool, 1964; Anon, 1972: Ben-Jaacov *et al*, 1978). Part II indicated that this may be partly due to high levels of applied N fertilizer making *T. speciosissima* susceptible to *Phytophthora cinnamomi*. However, even at the lowest level of added N in Experiment 3, there were still deaths. Seedling *T. speciosissima* are very variable in flower size and shape, leaf shape, plant size (Wills, 1959;Beadle *et al.*, 1976) and there is some evidence that wide variations in disease resistance exists between plants (Brackpool, 1964).

Part 1 demonstrated that *T. speciosissima* can be successfully propagated vegetatively. Some of the material which was produced from Experiment 1 (Part I) was planted out in the field. Among these there were some outstanding individuals which were both vigorous and produced large numbers of flowers (Plate 7.1). Despite the absence of a lignotuber there was extensive branching at ground level (Plate 7.2). However it was not known whether their vigour was due to environmental or genetic characteristics. This trial was designed to answer this question by propagating a number of these individuals and comparing their growth with seedlings.

Materials and Methods

On October 29, 1979, 40 one-year-old seedlings and 108 six month old cuttings of *T. speciosissima*[1] were planted in a sandy soil (yellow earth) at the Gosford Horticultural Research Station, Narara, NSW.

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1. Plants originated from Mangrove Mountain, N.S.W.

100 mm

Plate 7.1 Four year old vegetatively propagated plant of *Telopea speciosissima*. Note the large number of inflorescences.

Plate 7.2 Vegetatively propagated plant of *Telopea speciosissima* showing extensive branching at base despite the absence of a lignotuber. Branches arise from axillary buds.

The soil was previously uncultivated, about 1 m deep and composed of 85% fine sand. The cuttings were taken from 9 vigorous plants of *T. speciosissima* which had been initially propagated vegetatively, and the seedlings originated from seed gathered from the same 9 plants. The seedlings and cuttings had been planted 6 months previously into 150 cm pots filled with approximately 1 litre of sand and peat (1 : 1) with 0.15 g K_2SO_4, 1.2 g superphosphate, 3.0 dolomite limestone and 2.4 g $CaCO_3$ per litre. The plants were held for the first 5 months in a heated glasshouse (20°C minimum) and, for the last month before planting out, in a shade house (50% shade). The plants received 3.6 m moles N and 3.0 m moles K (as KNO_3 and NH_4NO_3) per pot per month. Both cuttings and seedlings developed extensive root systems with numerous proteoid roots.

At planting, seedlings and cuttings of approximately the same size were randomised and planted (Plate 7.3) 1 m apart in 2 rows, 2 m apart on the contour of a 30% slope. Watering was by individual microjet, each of which delivered about 10 l at each watering which was twice weekly for the first three months then once a week. Rainfall to the end of the experiment (16 October 1980) was 647 mm - approximately half the average rainfall which would have normally fallen during this period. Plants were mulched with 2.5 cm of shredded tree loppings and each plant received 100 g of blood and bone when transplanted.

After 12 months plant height was measured and the number of survivors of each treatment noted. The plants were retained for future assessment.

Number of survivors was statistically analysed using contingency tables (χ^2) and height data initially as a one factor analysis of variance (individual clones and seedlings) then the clones as a group were tested against seedlings (Steel and Torrie, 1960).

Plate 7.3 Typical examples of vegetatively propagated (left) and seedling (right) plants of *T. speciosissima* used in the experiment Growth of selected clonal material of *T. speciosissima* under field conditions. Note lack of lignotuber in the cutting-propagated plant.

Results

There was a large and significant difference (χ^2 = 27.6, 1 d.f., P < 0.005) between the clones (81%) and the seedlings (30%) with respect to plant survival (Table 7.1). However there was no significant difference (χ^2 = 2.6, 8 d.f., P > 0.05) between the individual clones.

There was a significant difference (P < 0.05) between (a) the heights of plants propagated by cuttings from the various mother plants and (b) between the clonal material as a whole and the seedlings (Table 7.1). Plants grown from mother plant No. 8 were significantly (P < 0.05) smaller in size than those from mother plants 1, 2, 3, 4, 5 and 7 (by the l.s.d. method - Steel and Torrie, 1960). The heights of the seedlings were also significantly less (P < 0.05) than the plants grown from these mother plants (Plates 7.4 and 7.5)

Discussion

Despite being grown under similar conditions, both in pots prior to planting out in the field and subsequently, a much higher proportion of plants propagated vegetatively from selected vigorous clones survived than seedlings grown from seed collected from the same source (81% compared to 30%). Both types of plants had well developed root systems and were initially of a similar size when planted.

The primary cause of the difference in survival was probably genetic in origin. *T. speciosissima* seedlings vary considerably in their physical characteristics and like *Grevillea*, which has a similar floral structure, are probably pollinated by birds (Burke, 1983) with a resultant genetic heterogenity of the seed.

Table 7.1 Percent survival and height of vegetatively and seedling-propagated *T. speciosissima* twelve months after transplanting into the field.

Plant type		Percentage of plants surviving	Av. height (cm)
Vegetatively propagated			
Clone No.	1	75	30.4
	2	83	32.5
	3	67	37.4
	4	83	32.7
	5	92	30.6
	6	67	22.2
	7	100	33.7
	8	83	15.2
	9	75	24.5
			s.e. (for n = 9.7) = 4.7
Av. for clones		81	28.8
			(s.e. = 1.6)
Seedlings		30	15.25
			(s.e. = 2.4)

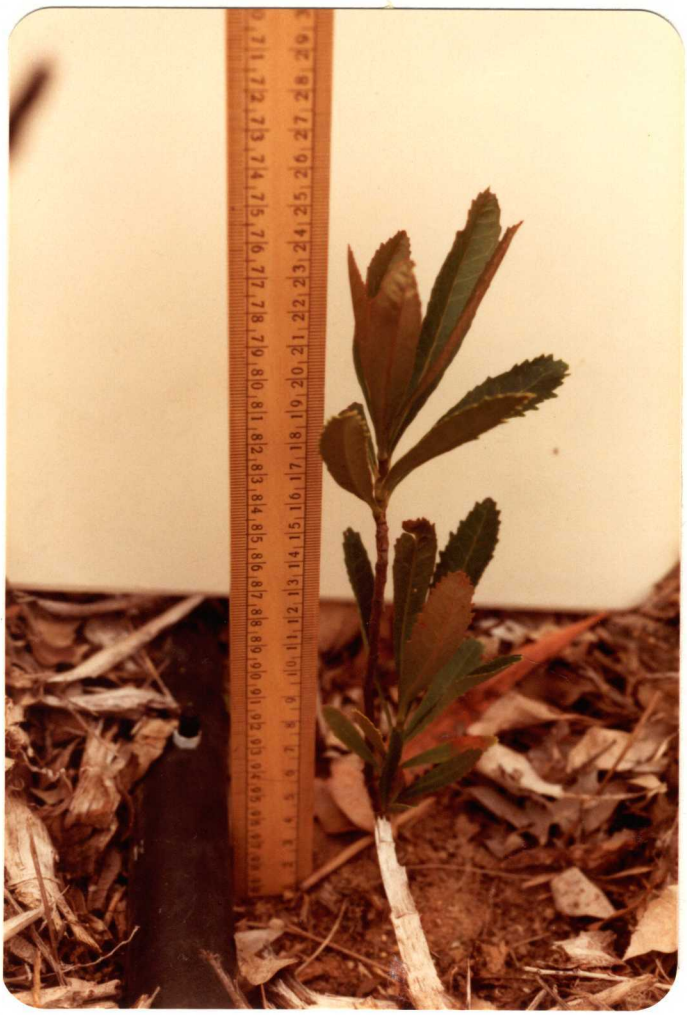

Plate 7.4 Typical example of a seedling of *Telopea speciosissima* 12 months after planting in the field. This was approximately the same size as the vegetatively propagated plant when planted in Plate 7.5.

Plate 7.5 Typical example of vegetatively propagated plant of *Telopea speciosissima* twelve months after planting in the field. Note branching near base despite lignotuber being absent. Branches arose from axillary buds below ground surface.

The cuttings, by virtue of the mother plants themselves being grown from cuttings, have been selected for their ability to survive, perhaps by increased plant vigour or increased resistance to diseases. A smaller percentage of seedlings have these desirable characteristics.

The cuttings were generally more vigorous than the seedlings as indicated by their greater height. One clone in particular, however, was less vigorous than the others. This indicates a need for further selection.

This trial has demonstrated that selected clonal material can be established with a high success rate when grown in a soil of low N status, high levels of organic matter, good drainage and adequate moisture. The vigour of selected clones was also generally greater than that of seedlings and their use may result in higher yields of flowers.

PART IV

POST HARVEST PHYSIOLOGY OF *TELOPEA SPECIOSISSIMA* INFLORESCENCES

CHAPTER 8 THE EFFECT OF PRESERVATIVE SOLUTIONS ON THE VASE LIFE OF *T. SPECIOSISSIMA* INFLORESCENCES (EXPERIMENT 6)

Introduction

HQC (8-hydroxyquinoline sulphate) has been shown to increase the vase life of a wide range of cut flowers by causing stomatal closure and inhibiting stem blockage. The site of blockage of water movement of cut inflorescences is in the stem, usually moving from the cut end upwards. Vegetative shoots can be used to test the effect of HQC on stem blockage. Leaves attached to the stem might be expected to provide an appropriate water sink. Paull *et al.*(1980) found that the foliage of cut *Protea* inflorescences was responsible for about 50% of the water loss by the leaves and inflorescence combined, whilst Carpenter and Rasmussen (1974) found that leaves of cut rose and carnation flowers were responsible for most of the water loss of the combined system.

Silver thiosulphate has also been shown to increase the vase life of a range of cut flowers. Its main mode of action in the inflorescence is to inhibit ethylene production and to reduce the sensitivity of flowers to ethylene. However it may also help to prevent stem blockage due to its fungicidal and bactericidal properties.

The senescence of both leaves and flowers can be accelerated by ethylene (Ables, 1973). Thus, if ethylene is important in the senescence of *T. speciosissima* inflorescences, silver salts may also slow the senescence of the leaves. In the absence of vascular blockage, senescence of the flower also reduces the ability of cut flowers (especially the leaves) to absorb water; this may be used to predict remaining vase life (Buys and Cours, 1980).

The supply of respirable substrates may also determine the vase life of flowers. Sucrose is often added to preservative solutions to increase this supply.

The general aim of Experiment (a) was to determine the effects of preservative solutions and stage of harvest on the vase life of *T. speciosissima* inflorescences. The specific aims were:

(a) determine the optimum stage at which to harvest inflorescences,

(b) determine the vase life of *T. speciosissima* inflorescences under standardised conditions,

(c) examine the effects of HQC (8-hydroxyquinoline citrate), sucrose and silver thiosulphate on the vase life of *T. speciosissima* inflorescences, and

(d) determine the effect of silver thiosulphate on the ethylene production of *T. speciosissima* inflorescences.

The aim of Experiment (b) was to examine the effect of HQC and silver thiosulphate on water uptake and stomatal closure of cuttings of *T. speciosissima*. The stems of the inflorescences or cuttings were not cut under water in these Experiments as it is not a normal practice in the commercial production of cut flowers.

Experiment (a) The effects of preservative solutions and stage of harvest on the vase life of *T. speciosissima* inflorescences,

Materials and Methods

Grading system

Inflorescences were initially graded according to the percentage of individual flowers that were open (perianth split and

style reflexed). Fully formed inflorescences with 0-5% of open flowers were assigned a score of 0 and those with 95-100% of flowers open were given a score of 5 with other inflorescences scored as in Table 8.1 and Plate 8.1. Inflorescences judged to have lost their decorative value for any reason (e.g. discolouration or wilting) were assigned a score of 6 regardless of how many of the individual flowers may have been open.

Effect of preservative solutions

(i) the effect of silver thiosulphate (0.02mM).

Forty inflorescences of *T. speciosissima* with 40cm stems and 5-6 leaves attached (average fresh weight 205g) were collected from an experimental plantation[1] at Somersby, New South Wales on 11th September, 1980. The inflorescences were fully formed but no styles had reflexed (i.e. a score of 0)

Immediately after cutting they were sealed in polythene bags to prevent moisture loss. Within 45 minutes of collection and, after removal from the plastic bags the basal 2cm of the cut stem was removed. Half the inflorescences were then stood to a depth of 10cm in distilled water (with a volume of 200ml per inflorescence) and the other half in 0.02mM silver nitrate plus 0.08 mM sodium thiosulphate solution in an air conditioned room ($20 \pm 1.5^{\circ}C$). Light (24 hr.) was supplied by cool white fluorescent tubes with an intensity of 960 lux (Gassen model 1.71-263 light meter, Gassen Instrument Company) and photosynthetic flux density of 14.6 μ moles$^{-1}m^{-2}$ (400-700mm) when measured with a model LI 1905 quantum meter (Lamba Instrument Company). The relative humidity varied between 65 and 80%.

Distilled water was used to replace the water utilized by the inflorescence, thereby allowing the maximum quantity of silver taken up by the inflorescence to be determined.

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1. Plants originated from Mangrove Mountain, N.S.W.

Table 8.1 Scores for stages in the development of *T. speciosissima* inflorescences.

Score	Stage of development (% styles reflexed)
0	0-5 (flowers fully expanded)
1	6-25
2	26-50
3	51-75
4	76-94
5	95-100
6	End of vase life. Inflorescences and/or leaves discoloured or wilted - no longer ornamental (n.b. - not all styles necessarily reflexed).

Plate 8.1a Inflorescence of *T. speciosissima* with a score of 0 (x 0.6)

Plate 8.1b Inflorescence of *T. speciosissima* with a score of 1 (x 0.6)

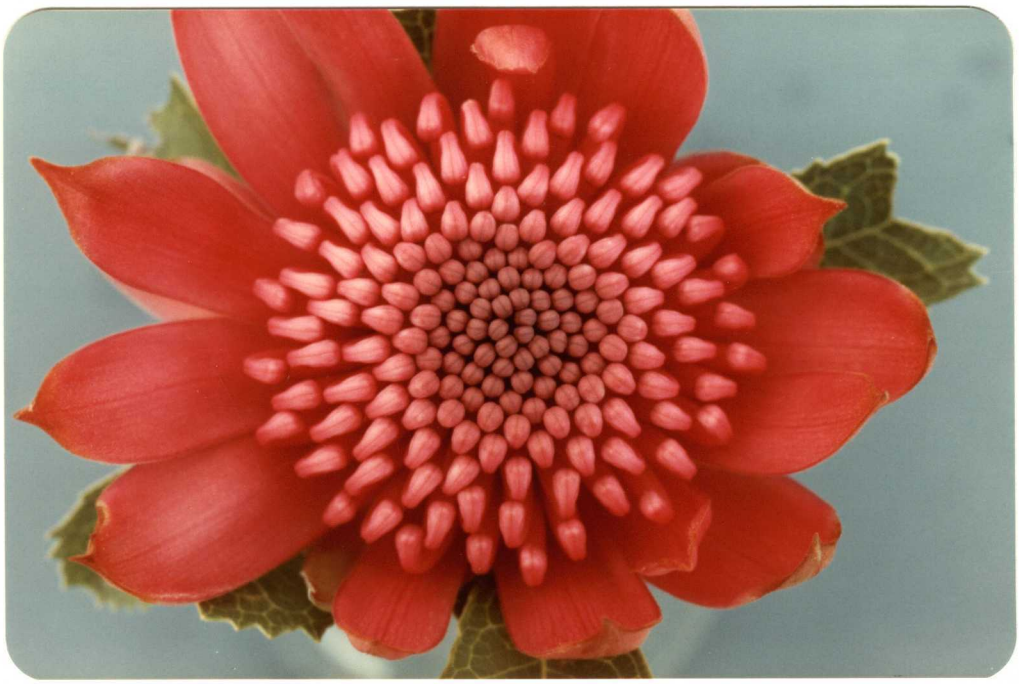

Plate 8.1c Inflorescence of *T. speciosissima* with a score of 2 (x 0.6)

Plate 8.1d Inflorescence of *T. speciosissima* with a score of 3 (x 0.6).

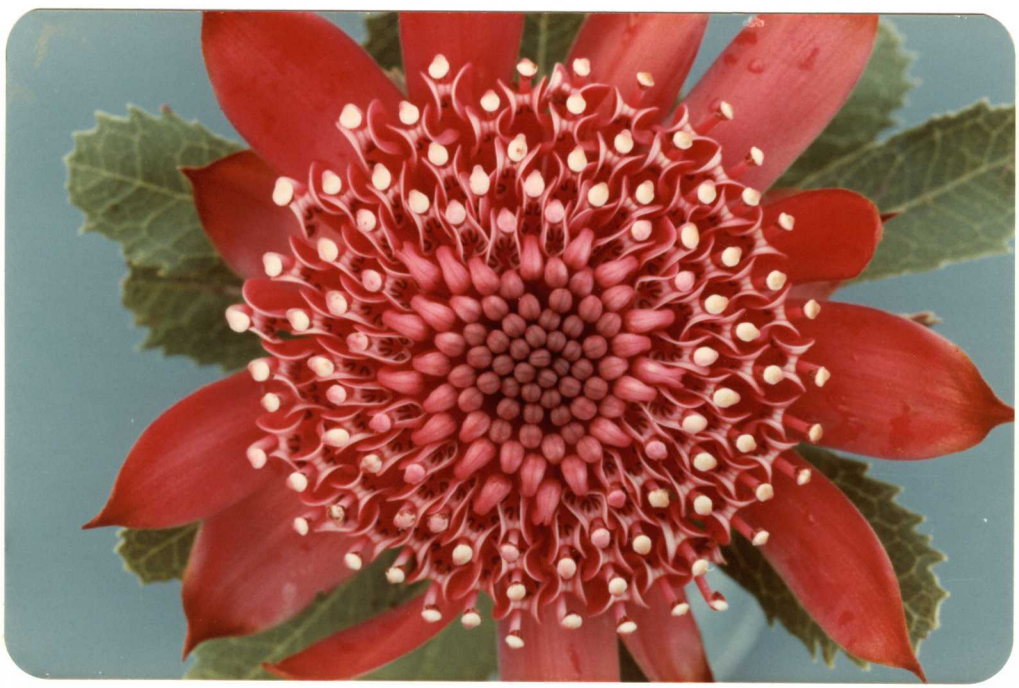

Plate 8.1e Inflorescence of *T. speciosissima* with a score of 4 (x 0.6)

Plate 8.1f Inflorescence of *T. speciosissima* with a score of 5 (x 0.6)

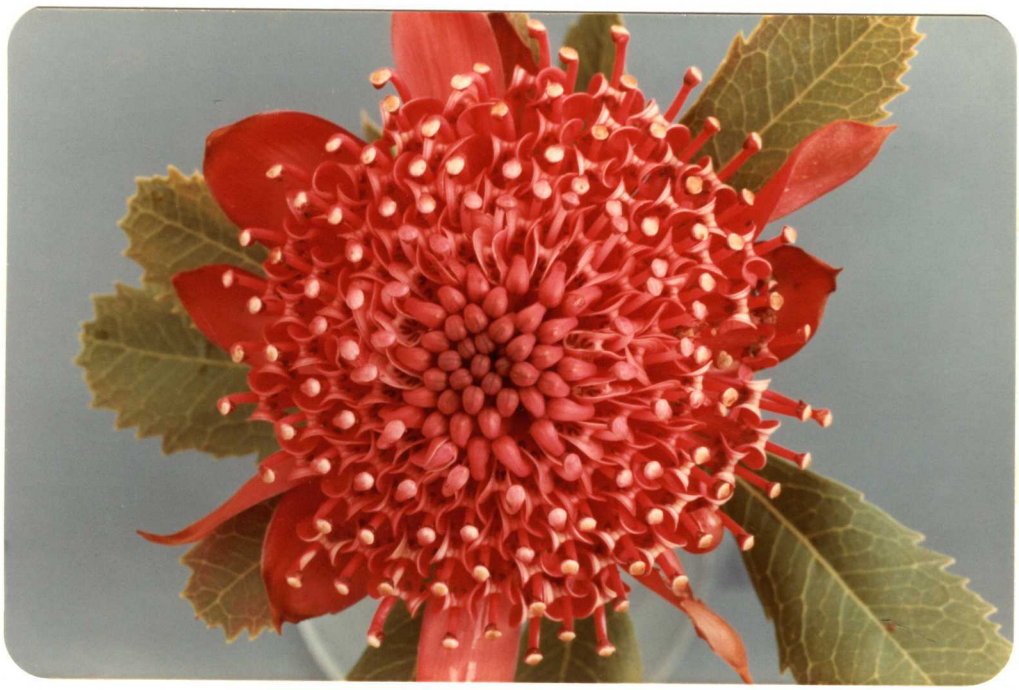

Plate 8.1g Inflorescence of *T. speciosissima* with a score of 5(x 0.6)

Plate 8.1h Inflorescence of *T. speciosissima* with a score of 6 (x 0.4). Note that not all the flowers are open. Leaves exhibiting symptoms of a silver toxicity.

The vase life of the inflorescences was determined by scoring them every second day according to the ratings in Table 8.1. When individual inflorescences reached stage 6 they were discarded. The two treatment means were compared using a t-test (Steel and Torrie, 1960)

(ii) Effect of silver thiosulphate (0.2mM Ag), HQC and sucrose.

On 14th September 1981, 84 inflorencences of *T. speciosissima* were collected from the same source and prepared in a similar fashion to those the Experiment a (i). Fourteen of the inforescences were then placed in each of the following solutions (200 ml per inflorescence) to a depth of 10cm in an air conditioned room similar to that used in Experiment a (i):-

(a) No added materials - (control).

(b) 27.3mM sucrose -(sucrose treatment).

(c) 0.2mM silver nitrate plus 0.8mM sodium thiosulphate - silver treatment).

(d) Combination of (b) and (c)- (sucrose plus silver treatment)

(e) 0.60mM 8-hydroxyquinoline citrate - (HQC treatment).

(f) Combination of (b) and (e) - (sucrose plus HQC treatment).

The vase life of the inflorescences was determined by subjectively scoring them according to the ratings in Table 8.1. Inflorescences were scored at the beginning of the Experiment and every second day until they reached stage 6, after which they were discarded.

Treatments were statistically compared as a one factor analysis of variance with treatment means being adjusted by the use of covariance to allow for the effect of the difference in initial scores on the vase life (Steel and Torrie, 1960). Regression analysis was used to derive an equation describing the relationship between the initial

score and vase life for the control (Steel and Torrie, 1960).

(iii) Development of immature inflorescences in a preservative solution

Immature inflorescences (bracts just reflexed) were treated as in (a) control and (f) sucrose plus HQC in Experiment a (i). There were 10 replicates (Plate 8.2).

(iv) Effect of silver thiosulphate on ethylene production.

Five inflorescences with an initial score of 0 were treated as in (a) control and (c) silver treatment of Experiment a (i). At stages with a score of 0, 4, 5 and 6 (Table 8.1) the amount of ethylene being produced by the inflorescences was determined by enclosing them individually in 10 l airtight translucent plastic containers for 24 hours in the air-conditioned room. Ethylene concentration in the enclosed air was measured using a Packard (R) model 427 gas chromatograph with a flame ionisation detector and using a 3.0mm stainless steel column, 74cm long filled with 80-100 Porapack N. The column was heated to 70°C and nitrogen gas was used. The practical limit of detection for this instrument is 0.01 v ppm.

Results

(i) Effect of silver thiosulphate

There was no significant difference ($P > 0.05$) between the vase life of the control (mean = 12.74 days) and those treated with silver thiosulphate (mean = 13.95 days, s.e. = 1,31).

(ii) Effect of silver thiosulphate, HQC and sucrose.

The linear relationship between initial score and vase life was responsible for a significant ($P < 0.05$) reduction in the residual sum of squares, i.e. vase life was affected by the initial score.

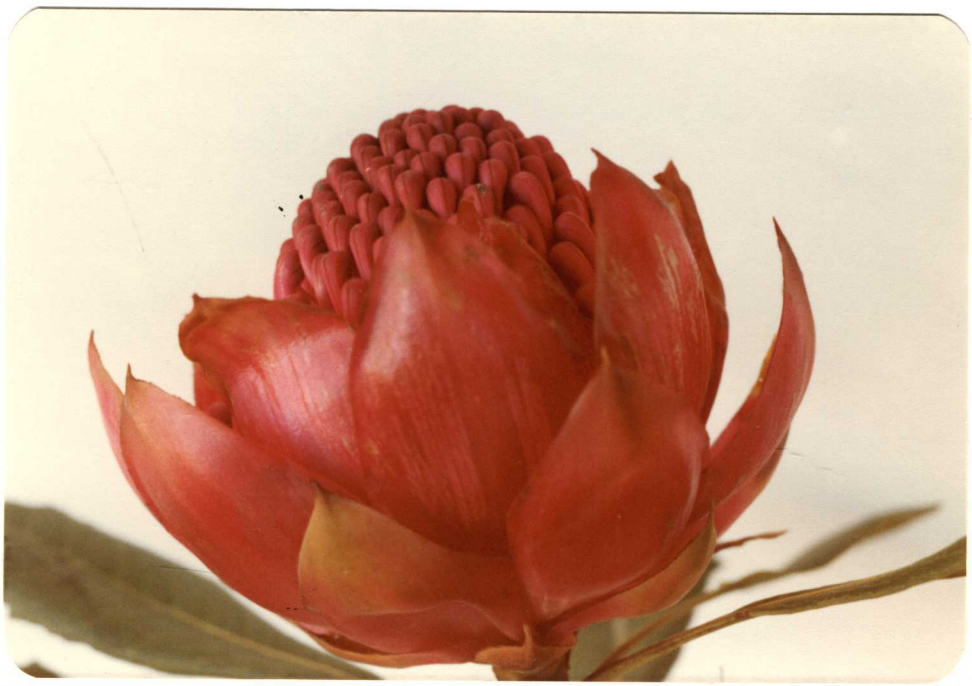

Plate 8.2 Immature inflorescence of *T. speciosissima* typical of those used in Experiment a(iii). (x 0.8)

The preservative solutions used did not increase the vase life of the inflorescences. Moreover, silver, sucrose or sucrose plus silver significantly ($P < 0.05$) reduced vase life (Table 8.2). Cloudiness and slime formation were evident in the sucrose solution.

(iii) Effect of maturity at harvest on vase life.

Immature inflorescences held in either water or a sucrose/HQC solution failed to develop to anthesis (i.e. the styles did not reflex) and they wilted after an average of 11.2 (s.e. = 0.5) days. There was no significant effect ($P > 0.05$) of treatment on time to wilting

All those inflorescences harvested at stage 0 or later reached stage 5 (i.e. 95-100% of the flowers reflexed) before losing their decorative value (stage 6).

The relationship between initial score and vase life (for the control) is given by equation 8.1:-

$$Y = 13.19 - 1.72X \quad (r = -0.85, P < 0.01)$$

where Y is the vase life in days, X is the initial score, $0<X<3$ and r is the correlation coefficient. The probability level quoted refers to the probability of the regression coefficient being less than 0. The higher order terms in X were not significant ($P > 0.05$). From equation 8.1, vase life is at a maximum (13.19 days) when the initial score of the inflorescences is 0 i.e. 0-5% of styles reflexed.

(iv) Ethylene production

The concentration of ethylene in the air-conditioned room was too low to be detected by the equipment used (<0.01v ppm). Similarly no ethylene was detected in the atmosphere of the sealed containers enclosing the *T. speciosissima* inflorescences. Therefore no inflorescences produced more than approximately 0.04 n l ethylene g^{-1} (fresh weight) hr^{-1}.

Table 8.2 Effect of various preservatives solutions on the mean (adjusted) vase life of *T. speciosissima* inflorescences.

Treatment (preservative solution)	Vase Life (days)
Control	11.9
sucrose	8.2
silver	7.0
silver plus sucrose	8.3
HQC	9.7
sucrose plus HQC	9.5

w (P = 0.05) = 2.51 (Tukey's *w* procedure - Steel and Torrie, 1960).

Experiment (b) The effect of HQC and silver on the transpiration rate of *T. speciosissima* cuttings.

Materials and Methods

Terminal stem cuttings 22cm long and trimmed to 5-6 leaves, with a total fresh weight of 10^{+}_{-} 0.5g were collected on 29th March, 1982. Cuttings were placed individually in test tubes (15 x 2cm) containing 50 ml of the test solutions. The tubes were placed at random in racks in an air-conditioned room with the same environmental characteristics as in Experiment (a). The solutions contained 0.00, 0.03, 0.06, 0.09 or 0.12mM Ag as silver nitrate and sodium thiosulphate in a 1:4 molar ratio or 0.075, 0.150, 0.300 or 0.600mM 8-hydroxyquinoline citrate in distilled water. The tubes were refilled daily to the original volume with distilled water and the volume required recorded. Twelve hours after the commencement of the Experiment, the diffusive resistance of the lowest leaf on each cutting was determined with a LI-COR, LI-1600 steady state porometer. There were 5 replicates for each treatment and 10 for the control (distilled water).

At the end of the Experiment leaf area was determined by a photo-electric area meter and the water loss (transpiration rate) was calculated on a leaf area basis. Five tubes containing only distilled water were used to estimate the change in potential evapotranspiration rate due to varying environmental conditions during the Experiment. All water loss data was multiplied by:

$$-\frac{\text{average evaporation from blank tubes on day x}}{\text{average evaporation from blank tubes on day 1}}$$

before analysis to correct for the varying environmental conditions.

Diffusive resistance data obtained with the steady state porometer

was analysed as a one factor analysis of variance. Regression analysis was used to statistically analyse the transpiration (water loss from tubes) data (Steel and Torrie, 1960).

Results

(a) Effect of HQC and silver thiosulphate on diffusive resistance.

Increasing concentrations of HQC or Ag^+ had no significant effect ($P > 0.05$) on the diffusive resistance of the cuttings (mean = 4.97 s cm^{-1}, s.e.= 0.25) indicating that silver thiosulphate and HQC do not cause stomatal closure in *T. speciosissima* at the rates tested.

(b) Effect of HQC and silver thiosulphate on transpiration.

The transpiration of untreated cuttings decreased with time (Figure 8.1). As the level of Ag^+ in the solution increased, their transpiration was reduced by a smaller amount over time (except for at 0.03mM). At the highest rate of Ag^+ (0.12mM) there was no significant ($P > 0.05$) reduction in transpiration with time. After 8 days however, leaves of cuttings at the two highest rates of Ag^+ began to discolour (became silver-grey) indicating that these rates are phytotoxic to *T. speciosissima*.

The transpiration of all cuttings treated with all rates of HQC did not decline with time nor was there any indication of phytotoxicity.

Discussion (Experiments (a) and (b))

Storage and transport of immature flowers in bud or partially opened offers many advantages. However in Experiment (a), immature *T. speciosissima* inflorescences placed in water or a solution of HQC and sucrose, which is commonly used to open buds, did not develop to maturity.

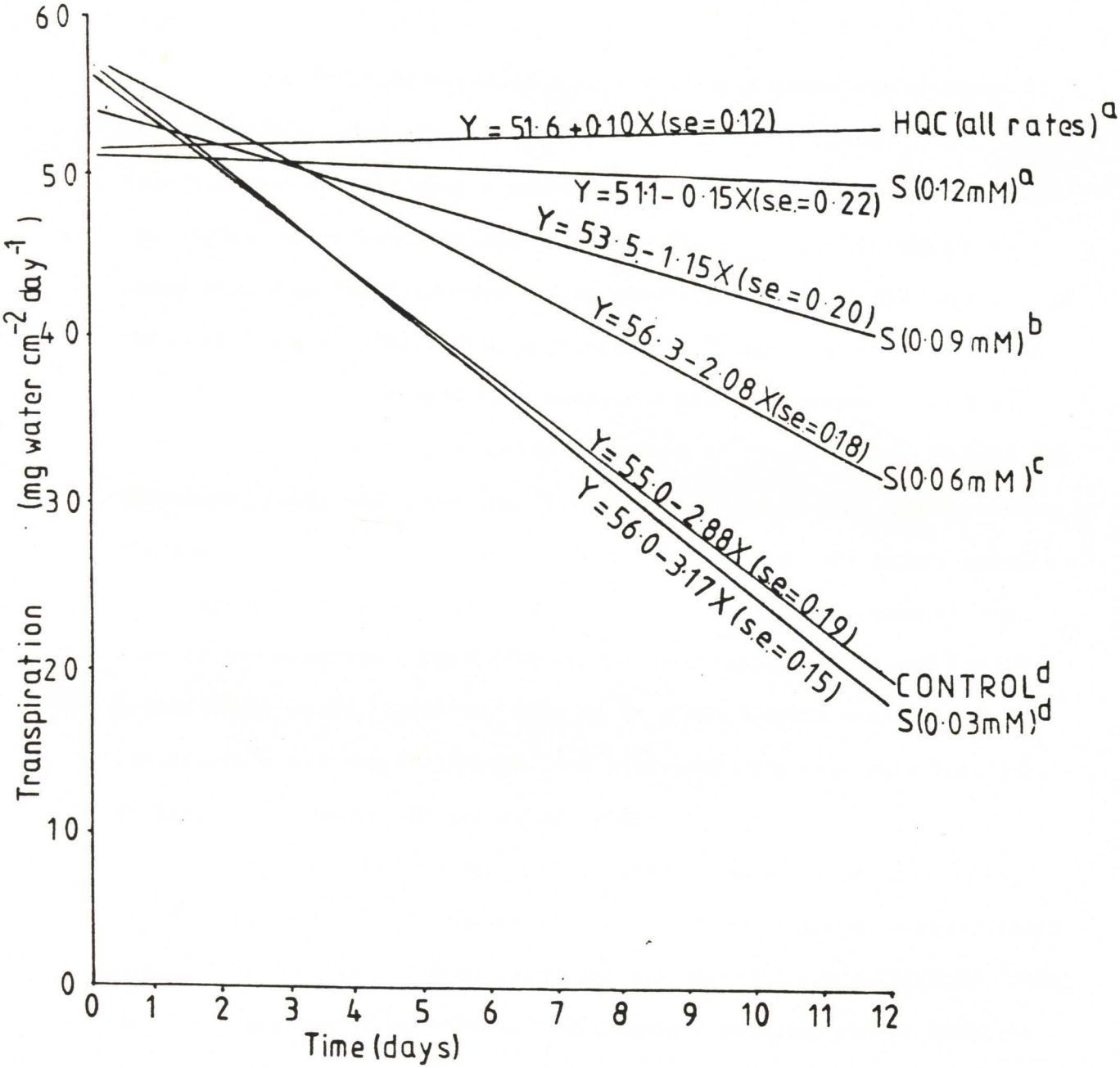

Figure 8.1 Effect of HQC and silver (s) concentration in the vase solution on the transpiration rate of *T. speciosissima* cuttings with time. The standard error (s.e.) given is that of the regression coefficient calculated from the raw data. The regression coefficients of rates with the same superscript are not significantly (P>0.05) different.

Therefore, until a method can be found which will allow post harvest development of the inflorescence of *T. speciosissima* they must, like those of *Leucospermum cordifolium*,be harvested fully formed (Jacobs, 1982).

The inflorescences of *T. speciosissima* harvested at stage 0 or later all reached stage 5 (95-100% of individual flowers open). Vase life was longest when flowers were harvested fully formed but with the styles not reflexed (stage 0). This differs from *Leucospermum* which should be harvested when three quarters or more of the styles have reflexed (Jacobs, 1982). Inflorescences of *T. speciosissima* harvested at stage 0 and not treated with chemicals have an average life of 13 days. This is generally comparable to that of treated roses (McLean and Rasmussen, 1980) and carnations (Sytsema, 1980, Mabir and Hanan, 1981), the two most commonly produced cut flowers and from this aspect the use of *T. speciosissima* for cut flowers could be considered commercially. However *Leucospermum cordifolium* (Watson and Parvin, 1973) and *Protea eximia* (Paull *et al*, 1980) may last up to 4 and 3 weeks respectively in preservative solutions although leaf blackening may end their vase life in less than 2 weeks, (Paull *et al*, 1980).

The vase life of the inflorescences was not increased by any of the treatments. All flowers probably produce ethylene, however their rates of production and dose responses are very different (Nichols, 1975). Of 12 species tested by Fischer (1950) only 3 produced enough ethylene to cause autotoxicity.

If *T. speciosissima* inflorescences produce ethylene it is at a very low level, even for flowers at the end of their vase life when production is usually the highest. This combined with no, or a negative, response to silver thiosulphate which inhibits the action of ethylene

suggests that *T. speciosissima* flowers belong to the group where an autotoxicity and a subsequent large reduction in vase life does not occur.

The failure of the 0.02 mM Ag to increase the vase life of the inflorescences in Experiment (a) (i) was not due to a failure of the silver to be absorbed. The phytotoxicity of the higher rates of silver salts applied, to both the inflorescence and leaves in Experiments (a)-(ii) and (b) indicates that silver ions were taken up from the solution. Silver thiosulphate was also not completely effective in preventing stem blockage except at rates at which it was phytotoxic to the leaves (Experiment b).

Where sucrose was added to the vase solution without an effective biocide (i.e. HQC) there was extensive microbial growth in the vase water as evidenced by slime formation and turbidity. The reduction in vase life caused by sucrose was probably due to stem blockage resulting in moisture stress in the inflorescence.

When HQC was added to the sucrose solution there was no significant decrease in the vase life, indicating that sucrose at the concentration used is not toxic to the inflorescences and HQC effectively inhibited microbial growth in the vase solution.

The lack of effect of sucrose plus HQC on vase life also indicates that the supply of respirable substrates in the inflorescence is not a limiting factor in the determination of their vase life, unlike the case of many other flowers.

The addition of HQC by itself to the vase solution had no detectable effect ($P > 0.05$) on the vase life of *T. speciosissima* inflorescences, although, even at the lowest concentration used (0.075 mM) it prevented a decline in the transpiration rate of the cuttings used

in Experiment (b). This indicates that HQC is completely effective in preventing stem blockage of *T. speciosissima* and that the decline of the transpiration rate of the control in Experiment (b) was due to stem blockage, rather than being associated with the senescence of the cutting. Stem blockage, however, was apparently not a limiting factor in the vase life of the untreated inflorescences in Experiment (a) though it may become a limiting factor if the vase life is extended by other means or in an environment with a greater potential evapotranspiration rate.

Silver thiosulphate and HQC however, had no significant effect on the transpiration rate of cuttings, 12 hours after application in Experiment (b).

Thus unlike the effect of HQC on a wide range of plant species, HQC and silver thiosulphate did not induce stomatal closure in *T. speciosissima*. It is unlikely that stomatal closure would have occurred later because it has been found that HQC usually affects the plant within a few hours of application and stomata usually reopen after 24 hours. This conclusion is further supported by the water uptake data for both HQC and silver thiosulphate at the beginning of the Experiment. Thus HQC and silver thiosulphate will not increase turgidity and therefore extend the vase life of *T. speciosissima* by causing stomatal closure.

Conclusions

Silver thiosulphate, HQC and sucrose, three chemicals commonly used to extend the vase life of flowers were not effective in increasing the vase life of *T. speciosissima* inflorescences, although HQC did prevent stem blockage and a subsequent 60% reduction in water uptake without

being phytotoxic. Thus HQC may extend the vase life of *T. speciosissima* inflorescences in environments with a greater potential evapotranspiration rate than that which occurred during the course of Experiment a (ii).

CHAPTER 9 EFFECT OF IPRODINE, CONDITIONING AND STORAGE AT 20°C AND 0.5°C ON THE VASE LIFE OF *T. SPECIOSISSIMA* INFLORESCENCES. (EXPERIMENT 7)

Introduction

Because of the short flowering season of *T. speciosissima* it would be commercially desirable to store the inflorescences for extended periods. In Experiment 6 it was shown that silver ions, HQC and sucrose, three chemicals commonly used to extend the vase life of flowers, were not effective in increasing the vase life of *T. speciosissima* inflorescences, although HQC did prevent stem blockage.

Flowers can often be stored for long periods at low temperatures (0-5°C) because their rate of respiration is reduced, but their subsequent vase life may be greatly reduced by extended cool storage.

The effective exploitation of most overseas markets using air transport will depend on holding inflorescences of *T. speciosissima* at about 20°C during transport (Qantas, personal communication). However, temperatures may rise above 20°C for short periods (Staby and Robertson, 1982; Lamont, 1983) during transportation, although use of ice and pre-cooling can reduce this to some extent. Up to 48 hours is required to transport the inflorescences by air to Europe (Lamont, 1983). To maintain plant turgor during transport, inflorescences are generally stored in plastic bags rather than water because of weight and space considerations (Paull *et al*, 1980).

A problem often encountered during transport at high temperatures and humidity is fungal development on the inflorescences (Lamont, 1983).

Any fungicide used should not reduce either their vase life or quality (e.g. by leaving residues).

Pre-conditioning, i.e. treatment before transport, can also affect their vase life. Water quality during this process can greatly influence the subsequent vase life of flowers. Use of HQC can also reduce or prevent subsequent stem blockage.

Flowers may also need to be stored after transport, especially if large quantities are transported at any one time.

The aim of Experiment (a) was to determine the effect of storage at 0.5°C on the subsequent vase life of *T. speciosissima* inflorescences at 20°C.

The aim of Experiment (b) was to examine the effect of various treatments on the vase life of *T. speciosissima* inflorescences after simulated transport. The specific aims were to examine:-

(a) the effect of Iprodine (Rovral$^{(R)}$), a fungicide, on the keeping quality of the inflorescences at high temperature (20°C) and humidity, especially with respect to residues and fungal infection.

(b) the effect of various conditioning treatments on the vase life of *T. speciosissima* inflorescences.

(c) the effect of storage for 48 hours at 20°C (to simulate air transport) on subsequent vase life

(d) the effect of storage in plastic bags for 7 days at 0.5°C after simulated air transport at 20°C.

Experiment (a) The effect of storage at low temperature (0.5°C) on the vase life of *T. speciosissima* inflorescences.

Materials and Methods

On 17th September 1981, inflorescences of *T. speciosissima* with an initial score of 0 (Table 8.1) were prepared as in Experiment (b). A total of 98 inflorescences (7 groups of 14) were placed in 6 containers with a 20cm depth of distilled water, covered with polythene plastic and stored at 0.5^{+}_{-} 0.5°C (except for the control group which was held at 20°C), After 0, 6, 10, 13, 15, 20, and 29 days a group of 14 inflorescences was transferred to an air-conditioned room, having the same environmental conditions as in Experiment 6, and the plastic covers were removed. Distilled water was used to maintain the water level in the containers. All inflorescences were scored as in Experiment 6.

Regression analysis was used to analyse the effect of time of storage on vase life (Steel and Torrie, 1960).

Results

The vase life of the inflorescences decreased as the storage period at 0.5°C was increased (Figure 9.1). Unlike in Experiment 6, all inflorescences did not pass through all score levels, especially those stored for longer periods, before reaching a condition which rendered them unacceptable as commercial cut flowers (stage 6). These flowers wilted and became discoloured before all their styles had reflexed.

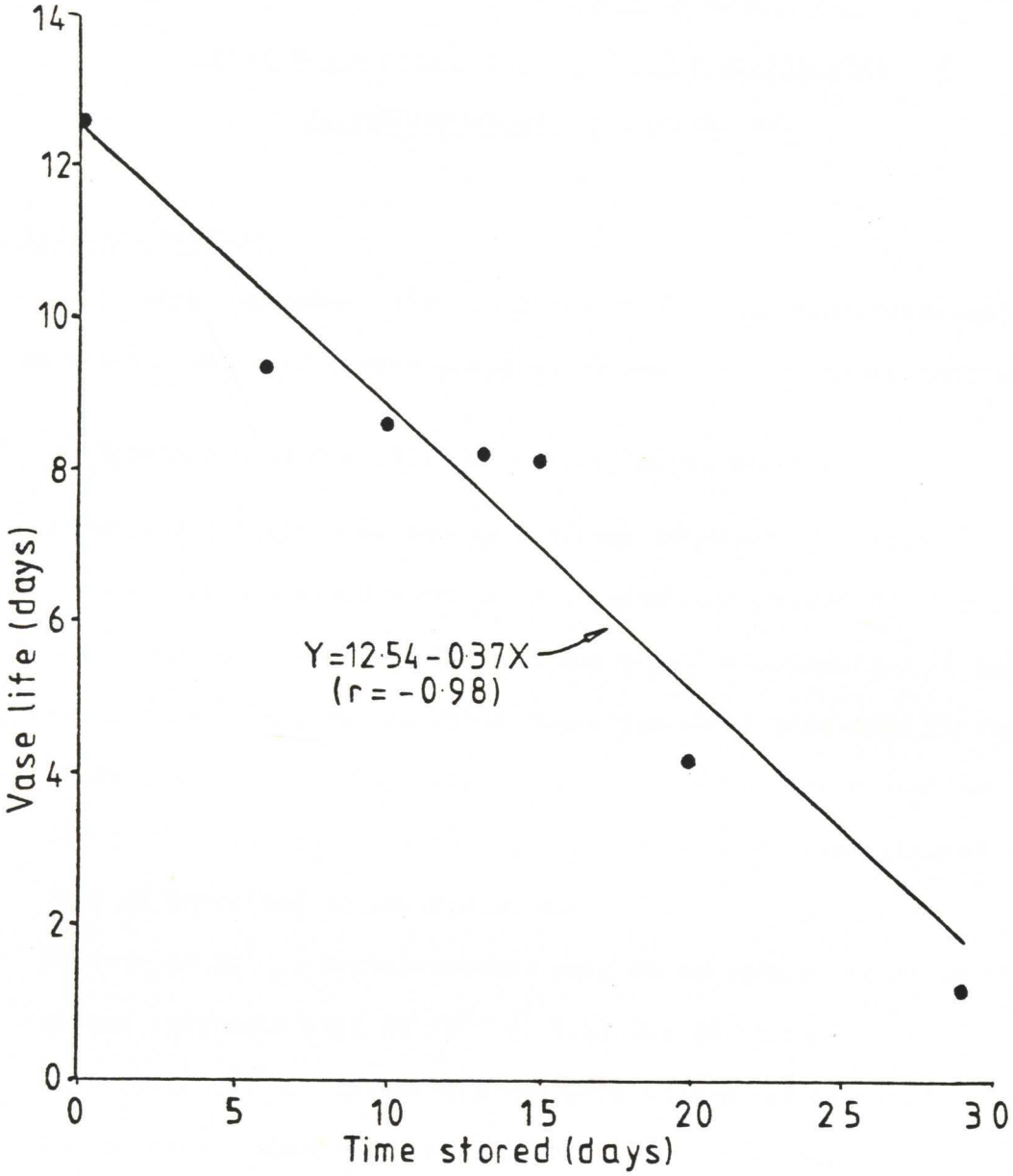

Figure 9.1 Effect of time of storage at 0.5°C on the subsequent vase life of *T. speciosissima* inflorescences. Data points represent means.

Experiment (b) Effect of Iprodine, conditioning solution, vase water and storage at 20 and 0.5°C on the vase life *T. speciosissima* inflorescences.

Materials and Methods

On 10th September 1982, inflorescences of *T. speciosissima* with an initial score of 0 were prepared as described in Experiment 6.

Treatments were applied in the following sequence:-

1. Fungicide. Inflorescences were either immersed in 0.25g/l Iprodine in distilled water or distilled water alone for 5 minutes.
2. Conditioning. The lower 20cm of the stems were immersed in tap water (analysis given in Table 9.1), tap water plus 4ppm Cl (W/V) as NaOCl, tap water plus 200ppm (W/V) HQC (8-hydroxyquinoline citrate) or distilled water for 3 hours in an air-conditioned room as described in Experiment 6.
3. Storage at 20°C. Inflorescences were stored out of water in sealed polythene bags at 20°C ($\pm$ 1.5) for 48 hours.
4. Storage at 0.5°C. Inflorescences were stored out of water in sealed polythene bags at 0.5°C ($\pm$ 0.5) for 7 days.
5. Vase water. Stems were immersed to a depth of 20cm in tap water, tap water plus 4ppm Cl (as NaOCl) or distilled water and held in an air-conditioned room as described in Experiment 6 (20 $\pm$ 1.5°C). The same scoring system as used in Experiment 6 was used to evaluate inflorescences.

Not all treatment combinations were used. The treatment combinations applied are given in Table 9.2. There were 15 replicates per combination. Analysis was initially as a one factor analysis of

Table 9.1 Chemical Analysis of Tap Water[1]

Parameter	Units
Electrical conductivity	0.15 mS/cm (25°C)
pH	7.46
Chloride	25 ppm
Sodium	8 ppm
Potassium	2.0 ppm
Calcium	13 ppm
Magnesium	2.8 ppm
Iron	0.2 ppm
Copper	0.08 ppm
Zinc	0.08 ppm
Manganese	0.04 ppm

(1) Analysis by B.C.R.I. Institute, N.S.W. Department of Agriculture

TABLE 9.2 Treatment combinations and vase life in Experiment 7

Treatment combination No.	Treatments applied: Fungicide	Conditioning solution	Storage (20°C)	Storage (0.5°C)	Vase solution	Vase life (days)
(a) No storage						
ai	yes	tap water	no	no	tap water	10.92[a](1)
aii	yes	tap water + HQC(3)	no	no	tap water	11.10[a]
aiii	yes	tap water +4 ppm Cl	no	no	tap water +4 ppm Cl	10.60[a]
aiv	yes	distilled water	no	no	distilled water	10.42[a]
av	no	distilled water	no	no	tap water	11.56[a]
Mean for ai and aii						11.01[x](2)
(b) Storage at 20°C						
bi	yes	tap water	yes	no	tap water	9.45[b]
bii	yes	tap water + HQC	yes	no	tap water	8.95[b]
biii	no	tap water	yes	no	tap water	discarded(4)
Mean for bi and bii						9.20[y]
(c) Storage at 20°C then 0.5°C						
ci	yes	tap water	yes	yes	tap water	7.43[c]
cii	yes	tap water + HQC	yes	yes	tap water	7.57[c]
ciii	no	tap water	yes	yes	tap water	discarded(4)
Mean for ci and cii						7.50[z]

1. Means with same superscripts (for a, b or c) are not significantly different (lsd = 1.42, P = 0.05).
2. Means with same superscript (for x, y or z) are not significantly different (lsd = 1.02, P = 0.05).
3. Addition of HQC had no significant effect on vase life (P > 0.05).
4. Discarded due to fungal growth making them commercially unacceptable.

variance then the data was partitioned. The effect of HQC and storage (no storage, storage at 0.5°C and storage at 20°C plus 0.5°C was examined as a two factor analysis of variance (i.e. treatments ai, aii, bi, bii, ci, cii).

Results

(a) Effect of conditioning and water quality in presence of fungicide.

There was no significant difference ($P > 0.05$) between the vase life of inflorescences held continuously in tap water (treat. ai), tap water plus 4 ppm Cl (treat. aii) or distilled water (treat. aiv).

HQC did not affect the vase life of *T. speciosissima* in this Experiment (Table 9.2).

(b) Effect of fungicide on keeping quality.

There was extensive growth of hyphae of *Botrytis cinerea* on inflorescences stored in plastic bags at 20°C and not treated with fungicide. These hyphae developed first on the stigmatic surface. Infested inflorescences were discarded after storage.

When inflorescences were treated with Iprodine, or were held continuously in the air-conditioned room, the infection did not develop. Application of fungicide did not affect the vase life of inflorescences held continously in the air-conditioned room. There was no visible residue of the fungicide on the inflorescences.

(c) Effect of storage at 20°C in plastic bags on vase life.

In the absence of fungal growth the vase life of inflorescences was 9.20 days after storage at 20°C for 2 days (Table 9.2) . Storage reduced the subsequent vase life by a period approximately equal to that of the storage time i.e. 1.90 days (lsd = 1.02, $P = 0.05$)

(d) Effect of storage for 7 days at 0.5°C after storage at 20°C in plastic bags for 2 days.

Storage at 20°C followed by 0.5°C significantly ($P < 0.05$) reduced the subsequent vase life of inflorescences by 3.60 days (Table 9.2) when compared to those continuously held in the air-conditioned room (no storage).

Discussion (Experiments (a) and (b)

The vase life of the inflorescences in Experiment (a) was reduced as the storage period at 0.5°C was increased. Cold storage generally reduces the subsequent vase life of cut flowers. Smith (1968) proposed at a 25% reduction in the vase life after storage of narcissus was acceptable to consumers while Rudnicki (1981) proposed that a reduction of 30% was acceptable for carnations which have approximately the same vase life as fresh *T. speciosissima* inflorescences. Using these criteria it can be concluded that *T. speciosissima* inflorescences may be stored at 0.5°C for 9-10 days (from Figure 9.1) without diminishing consumer acceptance. This storage time precludes sea shipment in refrigerated containers to most potential overseas markets (Jacobs, 1982). Air freight is the only alternative. Further research into storage methods is required if sea shipment is to become possible in the future.

Because of its cheapness and ready availability, tap water is usually used by flower producers for conditioning and storage of flowers (Jacobs, 1982). The same is true for consumers. The tap water used in this Experiment was of good quality (less than 200 ppm soluble salts)

and did not reduce the vase life of inflorescences. Use of good quality tap water in the presence of a fungicide instead of distilled or deionised water is thus unlikely to shorten the vase life of *T. speciosissima* inflorescences in commercial usage.

HQC may inhibit stem plugging especially where impurities are present in tap water or if the water becomes contaminated with micro-organisms. Its use had no effect on the vase life of inflorescences indicating that stem plugging did not reach a critical level, possibly due to the good quality of the tap water used.

During storage at 20°C in plastic bags, *Botrytis cinerea* developed extensively on inflorescences causing them to be discarded after 2 days. The fungus did not develop when inflorescences were held in an air-conditioned room at 20°C with a relative humidity of 65-80% suggesting that it was the high relative humidity within the bag that was responsible for its growth.

Botrytis cinerea may be controlled by the application of fungicides including Iprodine (Worthing, 1983). However these may leave objectionable residues when used at recommended rates. Iprodine was effective in suppressing the growth of *Botrytis cinerea on T. speciosissima* inflorescences stored in plastic bags in this Experiment. Application of the fungicide at half the recommended rate (Worthing, 1983) did not significantly ($P > 0.05$) reduce vase life when *B. cinerea* did not develop nor did it leave a visible residue. Treatment of inflorescences with a fungicide to control *B. cinerea* before air transport at temperatures of 20°C or greater would therefore appear to be essential.

In the absence of fungal infection, storage at 20°C reduced the subsequent vase life by a period approximately equal to that of the

storage time (Table 9.2).

Cold storage of inflorescences after shipment may be desirable to maintain a continuity of supply. Storage at 0.5°C for 7 days after simulated shipment at 20°C significantly ($P < 0.05$) reduced the vase life of the inflorescences. If the criteria of Smith (1968) and Rudnicki (1971) are applied again (i.e. a 25-30% reduction in vase life is permissible), total time at 20°C from harvesting to sale to the final consumer may not exceed 3-4 days. After simulated transport for 2 days at 20°C *T. speciosissima* inflorescences may also be stored for an additional 7 days at 0.5°C and still have an acceptable vase life.

CHAPTER 10 GENERAL DISCUSSION

Telopea speciosissima has proven to be unreliable in cultivation. Plants grown from seed are very variable with respect to vigour, leaf shape and flower size and shape. This study has shown that there also may be considerable variation among seedlings in their resistance to root-rotting diseases.

Selected clonal material used in Part III of this study had a much higher survival and growth rate than seedlings when planted into the field, indicating that they may be more resistant to root diseases. In Part II, where seedlings were planted in a yellow earth and supplied with a high N level, there was, despite the high mortality rate, a small percentage of apparently healthy plants. This further supports the conclusion that there may be considerable variation amoung seedlings in their resistance to root-rotting diseases.

Previous commercial experience suggested that it may be difficult to propagate *T. speciosissima* by cutting. However propagation of *T. speciosissima* from stem cuttings was highly successful in this study if they were collected during early spring at the commencement of vegetative growth. A success rate of about 95% was achieved if cuttings were treated with 2000 ppm IBA. Higher rates of IBA lead to a delayed toxicity. Application of benomyl was necessary to control infection of cutting by *Guignardia citricarpa*.

The performance of plants propagated by cuttings appears to be quite satisfactory in terms of growth rate and inflorescence production. The plant material used in Part III was derived from rooted cuttings produced during the experiment in Part I (vegetative propagation) and subsequently planted in the field. Many of these

plants were vigorous and produced large quantities of inflorescences. It would appear therefore that selected clones are a preferable means of establishing commercial plantings of waratahs to seedlings.

The waratah grows naturally in soils which are too infertile, especially in available N, P and K, for the normal growth of exotic pasture species and fruit trees. Many members of the *Proteaceae* which grow in similar soils have been reported to be sensitive to chemical fertilizers. The unreliability of *T. speciosissima* in cultivation has often been attributed to fertilizer excesses, particularly phosphorus. When grown in soil-less culture, however, this study demonstrated that the waratah showed increased growth when nitrogen and phosphorus were supplied at relatively high rates. Plants grown with high levels of nitrogen were more susceptible to *P. cinnamomi*. When grown in field soil supplemented with nitrogenous fertilizer at a rate of 482 m moles N per 18 kg of soil or greater there was a high mortality of *T. speciosissima*. It is probable the plant deaths were due to an increased susceptability to disease causing organism(s), possibly *P. cinnamomi*, rather than a direct fertilizer effect.

The response of *T. speciosissima* plants in the field to added N fertilizers may thus depend on the presence or absence of *P. cinnamomi*. This may also explain why various authors have advocated widely differing rates of fertilizer application for growing *T. speciosissima*.

There was, however, no response to additional K in the yellow earth, despite this soil having a very low level of available K. The application of K containing fertilizers is thus unlikely to be necessary in their commercial cultivation.

Proteoid roots are important to the plant in its natural habitat. However in cultivation their role will be limited because levels of available N and P which are optimal for vegetative growth will almost completely inhibit their formation.

The inflorescence of *T. speciosissima* is highly sought after as a cut flower. The vase life of freshly picked inflorescences (0-5% of styles reflexed) was shown to be 13 days at 20°C. Inflorescences can be stored at 0.5°C, without diminishing consumer satisfaction (i.e. a 25-30% reduction in vase life), for about 10 days. This is comparable to untreated commonly produced cut flowers (e.g. carnations, roses) and from this aspect their use could be considered commercially viable. However the vase life was shown not to be limited by the supply of respirable substrates, stem plugging or apparently ethylene. This precludes the use of commonly used floral preservatives to extend their vase life.

Most of the principal commercial cut-flower species are produced year round. This has been achieved by the development of perpetual flowering varieties (e.g. carnations, roses) or through the manipulation of growing conditions to control flowering (e.g. chrysanthemum, bulbs). The waratah has a relatively restricted flowering time when compared to other cut flowers and in its natural habitat (in any one location) flowering occurs over a period of about one month. This, combined with the short storage times that are possible, remains an impediment to its commercial exploitation. One possible means of extending the period that inflorescences are available is by having plantations in locations with different climates. This study demonstrated that flower times in two areas with different

climates within 100 km of each other may vary by one month. It may also be possible to select early and late flowering clones or manipulate the growing conditions to control flowering.

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APPENDIX - SUPPORTING PAPER.

The paper -

Worrall, R.J., 1976. Effects of time of collection, growing - conditions of mother plants and growth regulators on rooting of cuttings of *Telopea speciosissima* (Proteaceae). Scientia Hort. 5 : 153-160

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EFFECTS OF TIME OF COLLECTION, GROWING-CONDITIONS OF MOTHER PLANTS AND GROWTH REGULATORS ON ROOTING OF CUTTINGS OF *TELOPEA SPECIOSISSIMA* (PROTEACEAE)

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ABSTRACT

Worrall, R.J., 1976. Effects of time of collection, growing-conditions of mother plants and growth regulators on rooting of cuttings of *Telopea speciosissima* (Proteaceae). *Scientia Hort*, 5: 153—160.

Terminal stem cuttings of *Telopea speciosissima* were collected from two locations at monthly intervals and treated with indolebutyric acid (IBA) in combination with benomyl. Cuttings taken in the first half of spring, at the beginning of vegetative growth, gave the highest percentage of rooted cuttings and the greatest length of adventitious roots per rooted cutting. Cuttings taken from the environment most suited for the growth of the mother plants also produced the highest percentage of rooted cuttings, but their root length did not differ significantly between the two locations. Pre-treatment of cuttings with 4000 p.p.m. IBA (concentrated solution dip method) initially gave the highest percentage of rooted cuttings and longest root length, but there was evidence for a delayed toxicity to IBA applied at this level. Pre-treatment with 2000 p.p.m. IBA is therefore recommended.

Benomyl increased the percentage of rooted cuttings, but did not affect root length, possibly due to its fungicidal rather than cytokinin activity.

INTRODUCTION

Telopea speciosissima (Sm.) R.Br. (Proteaceae), native name "Waratah" (Fig.1), is increasing in importance as a cut flower in Australia. At present, most blooms are taken from plants growing in the wild, but the area under cultivation (approximately 4 ha) is expanding rapidly. Almost all of the cultivated plants have been grown from seed, partly due to difficulties experienced in rooting cuttings. Large variations in vigour, flower shape and size, and leaf shape exist between individuals established from seed.

Cuttings, as a means of propagation, have many advantages over seedlings. They allow selected desirable clones to be established (Rousseau, 1967) which flower in the first year after establishment. Seedlings take at least 2, but usually 3, years to flower (Parvin et al., 1973).

The cuttings of many proteaceous species are difficult to root, but respond well to indolebutyric acid (IBA), especially when applied as a solution

Fig.1. *Telopea speciosissima* (Sm.) R. Br.

(Rousseau, 1967). Cytokinins may also stimulate rooting of cuttings. Stimulation depends on the concentration of cytokinin used and the level of auxin present (Heide, 1965).

The time of year in which the cuttings are taken is an important factor in the production of adventitious roots and has been attributed to the physiological condition of the cutting-wood (Hartman and Kester, 1968).

This paper reports on the rooting of *T. speciosissima* cuttings as affected by the site (source) from which the cuttings were taken, the sampling-time,

IBA, and benomyl — which has both fungicidal and cytokinin-like properties (Skene, 1972).

MATERIALS AND METHODS

Vegetative *T. speciosissima* cuttings were taken from 2 sites: Mount Wilson (33 °61′ S, 150° 23′ E) in the Blue Mountains area and Mangrove Mountain (33 °19′ S, 151° 13′ E) in the Central Coast area of New South Wales, Australia. Cuttings from the Mount Wilson site (elevation 1066 m; average summer maximum 23 °C, minimum 12 °C; average winter maximum 10 °C, minimum 3 °C) were taken from *T. speciosissima* growing in the wild. At Mangrove Mountain (elevation 274 m; average summer maximum 24 °C, minimum 14 °C; average winter maximum 16 °C, minimum 6 °C) cuttings were taken from cultivated *T. speciosissima* which had received a total of 200 kg/ha superphosphate in the previous 2 years. The stems of cuttings from Mangrove Mountain tended to be thinner than those from Mount Wilson, possibly reflecting the higher density of plants in the cultivated area.

The treatments were:

(1) Source of cuttings: Cuttings were taken at each sampling-time from both the Mount Wilson and Mangrove Mountain sites.

(2) Sampling-time: The cuttings were taken at monthly intervals from March 1973 to February 1974. Twenty cm of stem with 5—6 leaves were taken and the condition and state of the terminal bud noted.

(3) Treatment with IBA: The cuttings were treated by the concentrated solution dip method (Hartman and Kester, 1968). The basal 5 mm of the cutting were dipped in a 50 % ethyl alcohol solution containing 0, 500, 1000, 2000 or 4000 p.p.m. (w/v) of IBA for 5 seconds. The 4000 p.p.m. rate of IBA was omitted for the first 3 sampling-times (March, April and May).

(4) Treatment with benomyl: Cuttings to be treated with benomyl were immersed in a 250 p.p.m. (w/v) benomyl solution for 30 seconds after the IBA solution had dried. The control cuttings were immersed in water.

Treatments with cuttings from Mount Wilson included 2 replications of 10 cuttings, but due to a shortage of available material those from Mangrove Mountain were not replicated.

After treatment, the cuttings were planted in pots containing perlite and placed in a glasshouse under an intermittent mist on a sand bed heated to 28 °C. The light intensity in the glasshouse was reduced by a 60 % shade cloth.

After 4 weeks the cuttings were removed from the pots and the number with roots longer than 5 mm counted. Those with roots shorter than 5 mm were replaced and re-examined after 4 weeks.

Prior to statistical analysis as an incomplete factorial, an arcsin $\sqrt{X}$ transformation was applied to all percentage data. When X was 100 %, 97.5 % was substituted for X and when X was 0 %, 2.5 % was substituted for X. All root length data were calculated on an average root length per rooted cutting-basis. A $\sqrt{(X + 0.375)}$ transformation was applied to these data.

RESULTS

Physiological condition of the cuttings. — During the winter months the mother plants were dormant except for the production of flower buds (Table 1). Growth and flowering in the spring began 1 month earlier at Mangrove Mountain than Mount Wilson. Growth also ceased later in the autumn at Mangrove Mountain.

Rooting of the Cuttings. — After 4 weeks growth there was no significant difference in the number of rooted cuttings collected from the 2 locations. However, after 8 weeks cuttings from Mangrove Mountain had a significantly higher percentage strike (51 %) than those from Mount Wilson (36 %). There were no significant interactions involving source of cuttings.

Benomyl-treated cuttings did not differ significantly from the control cuttings 4 weeks after collection. However, after 8 weeks the application of benomyl had significantly increased the percentage of rooted cuttings from 33 % to 58 %. Treated cuttings had minor infections of *Guignardia citricarpa* Kiely. On untreated cuttings the symptoms were far more severe; whole leaves became necrotic. There were no significant interactions involving the benomyl treatment.

TABLE 1

Physiological condition of the cuttings:
(A) Stems lignified and leaves mature. Terminal bud dormant with scales or flower developing from it.
(B) Commencement of leaf emergence from vegetative terminal bud.
(C) Distal 10 cm of cuttings new seasons growth. Leaves emerging from terminal bud.
(D) Cuttings of wood grown since previous spring. Stems lignifying. Leaves emerging from terminal bud.
(E) Cuttings of wood grown since previous spring. Stems and leaves semi-lignified. Terminal bud dormant with scales or flower developing from it.

Month of collection		Source of cuttings	
		Mangrove Mountain	Mount Wilson
Autumn	March	D	D
	April	E	EA
	May	A	A
Winter	June	A	A
	July	A	A
	August	A	A
Spring	September	AB	A
	October	BC Flowering	AB Flowering
	November	C	BC
Summer	December	CD	C
	January	D	CD

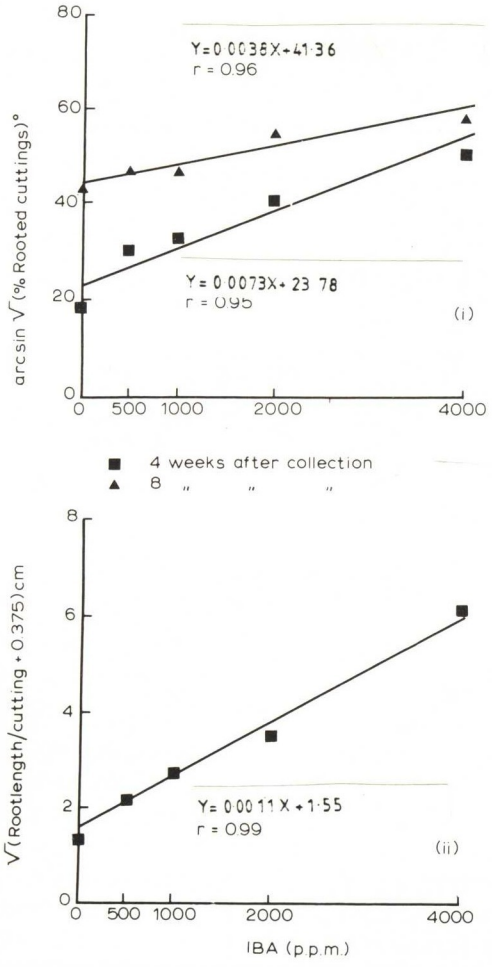

Fig.2. Effect of the level of IBA on (i) percentage rooted cuttings and (ii) root length per rooted cutting.

Increasing the level of IBA significantly increased both the percentage of rooted cuttings (Fig.2 (i)) and the root length (Fig.2 (ii)).

Following root initiation, the base of some cuttings became infected with a blackrot, which progressed both up the stem and along the roots. This effect was confined mostly to those treated with 4000 p.p.m. IBA. No organism isolated from affected cuttings could be associated with the symptoms.

Cuttings collected in September and October produced the highest percentage of rooted cuttings (Fig.3 (i)) as well as the greatest length of roots (Fig.3 (ii)). This period corresponds to the beginning of vegetative growth in spring (see Table 1). Another peak in root formation occurred in March just

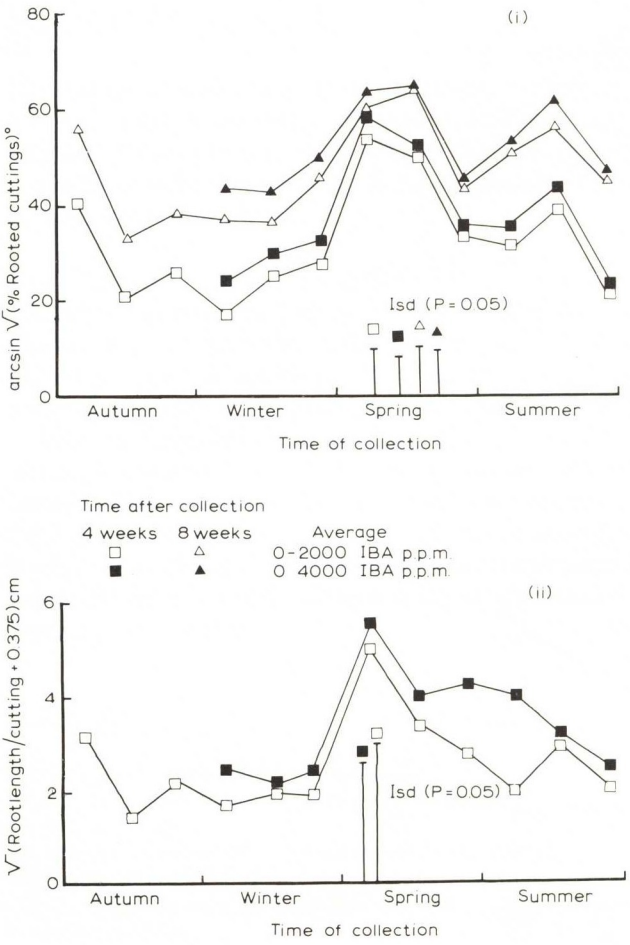

Fig. 3. Influence of month in which cuttings were taken on (i) percentage rooted cuttings and (ii) average root length per rooted cutting.

before vegetative growth ceased. The percentage of rooted cuttings and length of roots formed were poor when the plants were vegetatively dormant (e.g. winter).

A significant interaction occurred between the month of collection of cuttings and the level of IBA (Table 2). This was due to the differing responses of the cuttings taken at various times of the year to increasing levels of IBA.

DISCUSSION

The optimum time to take cuttings for both successful rooting and subsequent root growth coincided with the beginning of vegetative growth in spring.

TABLE 2

Effect of month of collection of cuttings and level of IBA on the percentage of rooted cuttings 4 and 8 weeks after collection

Month of collection		Transformed Data (degrees)									
		4 weeks after Collection Level of IBA (p.p.m.)					8 Weeks after Collection Level of IBA (p.p.m.)				
		0	500	1000	2000	4000	0	500	1000	2000	4000
Autumn	March	20	43	44	56		39	62	58	65	
	April	13	20	21	29		20	31	37	39	
	May	13	23	25	38		21	42	37	52	
Winter	June	13	13	18	21	51	22	32	43	50	63
	July	13	20	23	42	48	22	33	33	51	62
	August	13	21	26	42	51	35	37	45	62	63
Spring	September	31	50	55	71	77	39	56	66	76	71
	October	21	62	48	60	58	52	72	57	67	63
	November	15	35	37	39	41	23	49	48	45	47
Summer	December	19	34	33	30	49	42	54	48	48	58
	January	27	36	44	40	51	54	56	58	53	61
	February	15	13	22	22	30	41	44	41	48	48
		lsd ($P = 0.05$) = 15 lsd ($P = 0.01$) = 20					lsd ($P = 0.05$) = 16 lsd ($P = 0.01$) = 22				

Mother plants at Mangrove Mountain had a longer growing-period and a higher soil fertility than those at Mount Wilson. The better growing-conditions at Mangrove Mountain may have been responsible for the 15 % increase in the number of cuttings rooted from this area.

Since benomyl had no effect on either root length or on percentage cuttings rooted after 4 weeks, there is no evidence that it acted as a cytokinin. The 25 % increase in the percentage of rooted cuttings after 8 weeks is likely to result from its fungicidal properties, especially its ability to suppress *G. citricarpa* (Kiely, 1971).

In general the more concentrated the solution of IBA applied, the higher the percentage of rooted cuttings and the greater the root length. A large number of rooted cuttings, however, subsequently died, in particular those treated with 4000 p.p.m. IBA. Since a pathogen could not be associated with the deaths, there may have been a delayed toxicity to IBA, such as that exhibited by *Mandevilla* and some species of *Ficus* spp. (Hartman and Kester, 1968).

When delayed toxicity to IBA is taken into account, the optimum level of IBA in this experiment was 2000 p.p.m., even though at this level some delayed toxicity to IBA still occurred.

However, the response of the cuttings to IBA varied with the season. When the mother plant was actively growing such as in early spring, the cuttings responded better to low levels of IBA than when the plant was dormant, such as in mid-winter. Other factors such as rainfall also influenced root formation

by the cuttings. In the 2 months prior to collection of cuttings in February 1973 there was very little rainfall (4 cm) until 3 days before the cuttings were collected. Hence the plant was in a drought-induced dormancy. In contrast, the cuttings taken in March 1972, where in the previous 2 months there had been 50 cm of rainfall, produced a significantly higher percentage of rooted cuttings when IBA was applied than those taken in February 1973, despite lower ambient temperatures.

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