F01-1-Fl-024

J.L. Sharp. 1991. Condition of Florida grapefruit after exposure to vapor heat quarantine treatment. HortScience 26:42–44.
Miller, W.R., R.E. McDonald, T.T. Hatton, and M. Ismail. 1988. Phytotoxicity to grapefruit exposed to hot water immersion treatment. Proc. Fla. State Hort. Sci. 101:192–195.
SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 (ed.). SAS Institute Inc., Cary, N.C.
Sharp, J.L. 1985. Submersion of Florida grapefruit in heated water to kill stages of Caribbean fruit fly. *Anastrepha suspensa*. Proc. Fla. State Hort. Soc. 98:78–80.
Sharp, J.L. 1989a. Hot-water immersion appliance for quarantine research. J. Econ. Entomol. 82:189–192.
Sharp, J.L. 1989b. Preliminary investigation using hot air to disinfest grapefruit of Caribbean fruit fly immatures. Proc. Fla. State Hort. Soc. 102:157–159.
Sharp, J.L. and V. Chew. 1987. Time/mortality relationships for *Anastrepha suspensa* (Diptera: Tephritidae) eggs and larvae submerged in hot water. J. Econ. Entomol. 80:646–649.
Sharp, J.L., J.J. Gaffney, J.I. Moss, and W.P. Gould. 1991. Hot-air treatment device for quarantine research. J. Econ. Entomol. 84:520–527.
Smoot, J.J. and C.F. Melvin. 1965. Reduction of citrus decay by hot-water treatment. Plant Dis. Rpt. 49:463–476.
Spalding, D.H. and W.F. Reeder. 1985. Effect of hot water and gamma radiation on postharvest decay of grapefruit. Proc. Fla. State Hort. Soc. 98:207–208.
U.S. Department of Agriculture, Animal and Plant Health Protection Services. 1985. Plant protection and quarantine treatment manual. Sec. VI: T102, T106, and T107, Washington, D.C.

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Cold Storage of Selected Members of the Proteaceae and Australian Native Cut Flowers

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Additional index words. *Leucospermum* spp., *Protea* spp., *Leucadendron*, *Thryptomene calycina*, *Telopea speciosissima*, *Chamelaucium uncinatum*, *Verticordia* spp., *Anigozanthos* spp., postharvest physiology, vase life

Abstract. **Five members of the Proteaceae and 13 Australian native cut flower cultivars were stored for 35 days under standard conditions at 1C to assess their ability to withstand long-term storage and transport. *Protea cynaroides* L., *Leucadendron* 'Silvan Red', *Leucospermum* 'Firewheel', *Thryptomene calycina* (Lindl.) Stapf., *Telopea speciosissima* R. Br., and *Verticordia grandiflora* Endl. retained a vase life of at least 7 days after 21 days of storage. *Leucospermum cordifolium* Salisb. ex Knight, *Protea neriifolia* R. Br., *Chamelaucium uncinatum* 'Alba', *C. uncinatum* 'Purple Pride', *Verticordia monadelpha* Turcz., *Verticordia plumosa* (Desf.) Druce, and *Verticordia nitens* (Lindl.) Schau. suffered a decline in vase life ranging from 31% to 100% after 14 to 21 days of storage. Species of *Verticordia* and *Chamelaucium* were particularly susceptible to fungal infection. *Anigozanthos pulcherrimus* Hook. and the *Anigozanthos* cultivars Ruby Delight, Bush Harmony, Bush Haze, and Gold Fever all showed a significant reduction in vase life after 14 days of storage compared with unstored controls.**

Early research on the postharvest handling of cut flowers demonstrated the potential of dry storage in the rose, carnation, and chrysanthemum (Fischer, 1952; Hauge et al., 1947; Neff, 1939; Thornton, 1930). Recent studies have also concentrated on storage protocols for traditional flower crops (Goszczynska and Rudnicki, 1988; Hardenburg et al., 1986) The rapid expansion of the Australian cut flower industry has resulted in a marked increase in the export trade of Australian native cut flowers, particularly *Chamelaucium uncinatum* (Geraldton waxflower), *Anigozanthos* cultivars (kangaroo paw), and members of the Proteaceae. In the few storage trials conducted using these crops, the vase life of *Telopea speciosissima* (waratah) was reduced by 25% after 28 days of storage at 1C (Faragher, 1986), while vase life in *Anigozanthos rufus* and *Chamelaucium uncinatum* was significantly reduced by 14 days of storage at 1C (Joyce, 1988; Seaton and Joyce, 1989). *P. cynaroides* and *L. cordifolium* withstood 42 days of dry storage at 2C without reduction in vase life (Haasbroek et al., 1973; Ireland et al., 1967; Meynhardt, 1976). During a transient period of high airfreight costs, successful seafreight of cut *Protea* flowers from Cape Town, South Africa, to Rotterdam, Holland, was completed by several South African *Protea* growers in 1980 using refrigerated containers, but no data exist on storage and transport protocol (J. Wood, personal communication). Storage protocols and conditions differed widely between these trials, and in many cases the criteria for determining the end of vase life were not cited. The aim of this study was to assess the storage capacity of a wide range of commercial Australian native cut flowers and members of the Proteaceae family using specific vase life criteria and under a standard storage protocol. Cultivars that were suited to long-term dry storage were determined to be those that retained a vase life of at least 7 days after 21 days of storage.

Verticordia spp. were obtained from Western Australia and airfreighted to Knoxfield within 30 h of harvest. The *Chamelaucium* and *Anigozanthos* cultivars were harvested from commercial flower growers, cooled for 6 h, and transported dry for 6 to 12 h to the laboratory. All other flowers were harvested locally and transported in water, arriving at the laboratory within 3 h of harvest. Control, unstored flowers were sprayed with 1 g iprodione/liter (wettable powder, 50% a.i.; commercial name: Rovral, Rhone Poulenc, Melbourne, Australia) and placed in water at 1C for 24 h, then removed to 20C for vase life assessment. Stored blooms were thoroughly sprayed with 1 g iprodione/liter, allowed to dry, and kept at 1C for 24 h while standing in distilled water. Preliminary trials indicated that a pre- and poststorage treatment in water lasting at least 24 h was most effective (data not shown).

Flowers were bunched and tightly wrapped in two layers of newsprint and placed in low-density polyethylene bags (≈38 μm thick). These were then placed in fiberboard flower boxes (1030 × 370 × 160 mm) and stored in a room set at 1 ± 1C and 80% ± 5% relative humidity (RH). A nonstored control group was placed directly into the evaluation

Table 1. Criteria used for determining end of vase life.

Genus	Termination of vase life
Leucospermum	First sign of wilting styles
Protea	Wilting styles and/or first sign of leaf blackening
Leucadendron	Desiccation of leaf tips
Thryptomene	50% of flowers closed
Telopea	Perianth wilting and/or blue color
Chamelaucium	First sign of flower closing and abscission
Verticordia	First sign of flower closing and abscission and/or fading
Anigozanthos	Wilting flowers and/or fading

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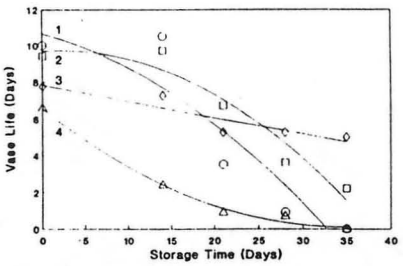

Fig. 1. Fitted curves representing changes in vase life after dry storage at 1C in members of the Proteaceae. 1) *L. cordifolium* (○); Y = 10.66 − 0.129X − 0.00596X²; Adj. R^2 = 60.4. SE = 0.128X; 0.00353X². 2) Firewheel (□); Y = 9.68 + 0.033X − 0.00756X²; Adj. R^2 = 39.4. SE = 0.137X; 0.00378X². 3) *P. cynaroides* (◇); Y = 7.83 − 0.0883X; Adj. R^2 = 17.9. SE = 0.0389X. 4) *P. neriifolia* (△); Y = 6.58 − 0.374X + 0.00542X²; Adj. R^2 = 76.4. SE = 0.0508X; 0.0014X². Where Y = vase life (days); X = storage time (days).

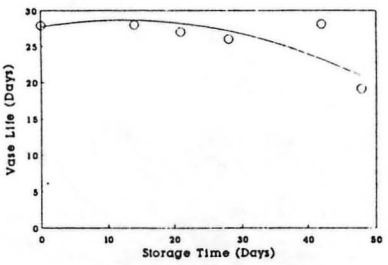

Fig. 2. Fitted curve representing change in vase life in *Leucadendron* 'Silvan Red' after dry storage at 1C. Y = 27.72 + 0.156X − 0.00626X²; Adj. R^2 = 25.2. SE = 0.182X; 0.00321X². Where Y = vase life (days); X = storage time (days).

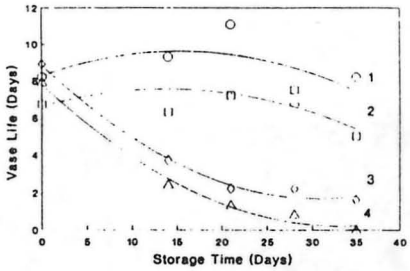

Fig. 3. Fitted curves representing changes in vase life in selected Australian native cut flowers after dry storage at 1C. 1) *T. speciosissima* (○); Y = 8.273 + 0.1744X − 0.00566X². Adj. R^2 = 8.3. SE = 0.0847X; 0.00234X². 2) *T. calycina* (□); Y = 6.622 + 0.1341X − 0.00482X². Adj. R^2 = 19.0. SE = 0.0552X; 0.00153X². 3) *C. uncinatum* 'Alba' (◇); Y = 8.847 − 0.4657X + 0.00756X². Adj. R^2 = 81.7. SE = 0.0489X; 0.00135X². 4) *C. uncinatum* 'Purple Pride' (△); Y = 7.8 − 0.4582X + 0.0069X². Adj. R^2 = 83.8. SE = 0.0478X; 0.00132X². Where Y = vase life (days); X = storage time (days).

room. After storage, the flowers were unwrapped, the stems recut and rehydrated at 1C in distilled water for 24 h, with flower heads covered with a polyethylene bag similar to that used in packing to increase relative humidity in the air surrounding the flower heads. Vase life was evaluated at 20C and 55% to 65% RH under constant light (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) supplied by cool-white fluorescent lamps. Criteria used to specify the end of vase life for each species and cultivar were defined (Table 1).

Each treatment (storage time) consisted of 10 replicate stems, and each storage trial was repeated twice. Regression analysis was performed on the data. Those stems infected with fungal growth during storage were arbitrarily assigned a vase life of zero, and these values were included in the calculation of mean vase life.

Proteaceae. Leucadendron 'Silvan Red', *Leucospermum* 'Firewheel', and *Protea cynaroides* retained a vase life of at least 7 days after 21 days of storage (Figs. 1 and 2). The vase life of 'Silvan Red' had decreased by only 11% (25 days compared with 28 days in unstored controls) after 28 days of storage (Fig. 2), and storage was therefore continued for an additional 21 days. 'Silvan Red' maintained a commercially acceptable vase life of 19 days even after 49 days of storage (Fig. 2).

Poststorage vase life of *Leucospermum cordifolium* declined rapidly after 14 days of storage, mainly due to fungal infection (data not shown). A more effective anti-fungal treatment than we used probably would dramatically improve poststorage vase life in this flower. Preliminary trials with *L. cordifolium* indicated that stems not infected with fungal rot during storage had a vase life of at least 7 days after 21 days of storage.

Cold storage-induced leaf blackening after 14 days of storage in *P. neriifolia* resulted in a short poststorage vase life. Storage-induced leaf blackening was not observed in *P. cynaroides.*

Australian native cut flowers. Generally, Australian native cut flower species stored in these trials did not withstand the rigors of dry storage as well as members of the Proteaceae family (Figs. 3, 4, and 5). However, vase life in *Thryptomene calycina* and *Telopea speciosissima* did not change significantly after 21 days of storage (11 and 7 days, respectively; Fig. 3).

Verticordia grandiflora maintained a vase life of 10 days after 21 days of storage (Fig. 4), after which vase life declined severely, whereas the vase life of all other species of *Verticordia* declined significantly after 14 days of storage. No regression analysis was performed on *V. monadelpha* and *V. plumosa* as no stems survived after 14 days of storage.

Significant floral abscission occurred in *T. calycina* after 28 days of storage (≈15% to 20% of flowers). As there was no further abscission during vase life assessment, these stems were rated in a similar manner as control stems. Observations during preliminary trials indicated that abscission became a major problem during storage only when flowers are harvested late in the season and in full flower.

The vase life of stored *T. speciosissima* was 8 days after 35 days of storage, similar to the vase life of unstored control stems (Fig. 3). Faragher (1986) reported that waratah vase life was reduced by 50% (from 6 to 3 days) if stems were stored at 4C, or if flowers were stored unwrapped, indicating the importance of low temperature (1C) and the need to maintain flower hydration during storage with adequate wrapping.

Quality in both *C. uncinatum* 'Alba' and 'Purple Pride' declined rapidly after 14 days of storage (Fig. 3). Stems of *C. uncinatum* were stored with a similar reduction in vase life by Seaton and Joyce (1989). *C. uncinatum* suffered from fungal attack (identified as *Botrytis cinerea* Pers.) in these trials, despite a prestorage spray with 1 g iprodione/liter, resulting in poor poststorage vase life. Fungal infection was also prevalent in all *Verticordia* spp. and kangaroo paw cultivars after 21 days of storage. When present, it effectively ended vase life immediately after storage. The *Verticordia* spp. were not treated with an anti-fungal agent before air transport from Western Australia to Victoria, and conditions during transit (high temperature, high

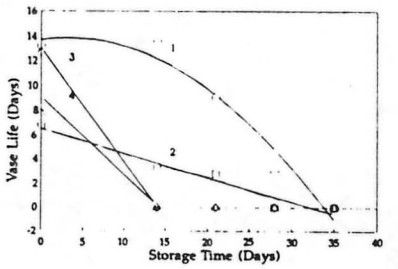

Fig. 4. Fitted curves representing changes in vase life in *Verticordia* spp. after dry storage at 1C. 1) *V. grandiflora* (○); Y = 13.67 + 0.1049(X) − 0.01499(X²); Adj. R^2 = 92.9. SE = 0.0587(X); 0.0016(X²). 2) *V. nitens* (□); Y=6.503 − 0.2012(X); Adj R^2 = 72.0. SE = 0.0178(X). 3) *V. monadelpha* (◇). No regression equation. 4) *V. plumosa* (△). No regression equation. Where Y = vase life (days); X = storage time (days).

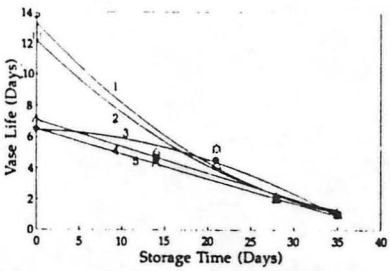

Fig. 5. Fitted curves representing vase life in kangaroo paw cultivars after dry storage at 1C. 1) 'Gold Fever' (○); Y = 13.3 − 0.578X + 0.00659X². Adj. R^2 = 83.5. SE = 0.0757X; 0.00208X². 2) *A. pulcherrimus* (□); Y = 12.17 − 0.521X + 0.0059X². Adj. R^2 = 72.0. SE = 0.0941X; 0.0026X². 3) 'Bush Harmony' (◇); Y = 6.45 − 0.01X − 0.0042X². Adj. R^2 = 69.5. SE = 0.0498X; 0.00138X². 4) 'Bush Haze' (△); Y = 7.1 − 0.1726X; Adj. R^2 = 80.8. SE = 0.012X. 5) 'Ruby Delight' (▽); Y = 6.53 − 0.162X; Adj. R^2 = 73.6. SE = 0.014X. Where Y = vase life (days); X = storage time (days).

relative humidity) possibly were conducive to fungal attack. Subsequent spraying with 1 g iprodione/liter was not sufficient to prevent further fungal infection.

The evaluation of other anti-fungal agents and spraying/dipping techniques has established the anti-fungal potential of a mixture of 1 g iprodione/liter and 1 g mancozeb/liter (wettable powder, 80% a.i.; commercial name: Mancozeb, suggested by D. Joyce; data not shown). An effective anti-fungal treatment that can be applied immediately after harvest to improve the storage performance of *C. uncinatum* and *Verticordia* spp. possibly will be developed.

Kangaroo paw cultivars with a long control vase life (*Anigozanthos pulcherrimus* and the cultivar Bush Fever) suffered a substantial reduction in vase life after 14 days of storage (Fig. 5), leaving all kangaroo paw cultivars with a vase life of <7 days after 14 days of storage. Seaton and Joyce (1989) reported a dramatic decrease in vase life when *Anigozanthos rufus* cut flowers were stored for 2 weeks at 0C. Vase life significantly increased in unstored kangaroo paws pulsed with sucrose solutions of up to 30% (Carter et al., 1989; Manning et al., 1989), and it is possible that pre- and poststorage treatment with a sucrose solution might extend vase life significantly.

The vase lives cited in this study represent a minimum value. Further improvements of this storage protocol, including more advanced packaging techniques and the use of solutions containing a germicide and sucrose in the precooling and rehydration phases, should improve the length of storage possible and the condition of flowers after storage.

Our results suggest that *Leucospermum* 'Firewheel', *Protea cynaroides*, *Leucadendron* 'Silvan Red', *Thryptomene calycina*, *Telopea speciosissima*, and *Verticordia grandiflora* can be stored for at least 21 days and retain a commercially acceptable vase life of at least 7 days. Vase life of stored *Leucospermum cordifolium*, *Chamelaucium uncinatum* 'Alba' and 'Purple Pride', *Verticordia monadelpha*, *Verticordia plumosa*, *Verticordia nitens*, *Anigozanthos pulcherrimus*, and the *Anigozanthos* cultivars Ruby Delight, Bush Harmony, Bush Haze, and Gold Fever declined significantly compared with unstored controls. Australian native species were susceptible to fungal attack during storage. Effective anti-fungal treatments may extend the storage period and vase life of these species.

Literature Cited

Carter, E.M., D.C. Joyce, and T.J. Enright. 1989. Pulsing native Australian cut flowers with sugar. Proc. Conf. Production and Mktg. Austral. Flora, 13–14 July 1989, Univ. of Western Australia, Perth.

Faragher, J.D. 1986. Effects of cold storage methods on vase life and physiology of cut waratah inflorescences (*Telopea speciosissima*, Proteaceae). Scientia Hort. 29:163–171.

Fischer, C. 1952. Long-term holding of cut flowers. Proc. Amer. Soc. Hort. Sci. 61:585–592.

Goszczynska, D.M. and R.M. Rudnicki. 1988. Storage of cut flowers. Hort. Rev. 10:35–64.

Haasbroek, F.J., G.G. Rousseau, and J.F. de Villiers. 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R. Br., *Protea longiflora* Lamarck and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5:33–42.

Hardenburg, R.E., A.E. Watada, and C.Y. Wang. 1986. The commercial storage of fruits, vegetabls, and florist and nursery stocks. U.S. Dept. Agr., Agr. Hdbk. 66.

Hauge, A., W. Bryant, and A. Laurie. 1947. Packaging of cut flowers. Proc. Amer. Soc. Hort. Sci. 49:427–432.

Ireland, J.P., J.T. Meynhardt, and J.M. Strauss. 1967. When Proteas become sailors—treatment before shipping. Farming in South Africa: Dept. Agr. Tech. Serv., Pretoria, S. Africa, Sept. 1967. p. 33–35.

Joyce, D.C. 1988. Postharvest characteristics of Geraldton Waxflowers. J. Amer. Soc. Hort. Sci. 13:738–742.

Manning, L.E., D.C. Joyce, and B.B. Lamont. 1989. Postharvest handling of kangaroo paws. Proc. Conf. Production and Mktg. Austral. Flora, 13–14 July 1989, Univ. of Western Australia, Perth.

Meynhardt, J.T. 1976. Proteas—picking and handling. Farming in South Africa; Flower, Ornamental Shrubs and Trees Series no. B5/1976 Dept. Agr. Tech. Serv., Pretoria, S. Africa.

Neff, M.S. 1939. Problems in the storage of cut carnations. Plant Physiol. 14:271–284.

Seaton, K.A. and D.C. Joyce. 1989. Cold storage of Geraldton Wax, kangaroo paw and Banksia. Proc. 5th Austral. Agron. Conf., Sept. 1989, Univ. of Western Australia, Perth. p. 532.

Thornton, N.C. 1930. The use of CO_2 for prolonging the life of cut flowers with special reference to roses. Amer. J. Bot. 17:614–626.

HortScience 26(11):1397–1400. 1991.

Forcing Irradiance, Temperature, and Fertilization Affect Quality of 'Gloria' Azalea

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Additional index words. longevity, postharvest, light, nutrition, *Rhododendron*

Abstract. **Dormant-budded 'Gloria' azaleas (*Rhododendron* sp.) were used to observe the effect of forcing irradiance, temperature, and fertilization on postproduction performance after flower bud dormancy had been broken. Four experiments were conducted during forcing, the treatments for each experiment were: Expt. 1, three forcing irradiances (200, 460, and 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and three postproduction irradiances (4, 8, and 16 $\mu mol \cdot m^{-2} \cdot s^{-1}$); Expt. 2, three forcing irradiances (320, 560, and 1110 $\mu mol \cdot m^{-2} \cdot s^{-1}$); Expt. 3, three controlled day/night temperatures (18/16C, 23/21C, and 29/27C); Expt. 4, fertilizer applied for 7, 14, or 28 days at either 150 or 300 mg N/liter (12% nitrate, 8% ammoniacal) 20N–4.8P–16K soluble fertilizer at every watering, control plants did not receive fertilizer. Days to harvest (time until plants had eight individual open flowers) was less at the high forcing irradiances and temperatures and when fertilizer was applied during forcing. Flower color was less intense at the low forcing irradiance levels, high temperatures, and when duration of fertilization was prolonged and concentration was high. There were more open flower inflorescences at week 2 of postproduction at high forcing irradiance levels, but their number was not affected by forcing temperature or fertilization. Postproduction longevity was shorter when forcing was at 29/27C (day/night) and when plants were fertilized for 28 days at 300 mg N/liter, but was not affected by forcing or postproduction irradiance.**

Irradiance levels, temperature, and fertilization have direct effects on development and postproduction performance of floriculture crops. High irradiance levels have been observed to shorten time to flowering in 'Reinhold Ambrosius' and 'Knut Erwén' azaleas (Bodson, 1983). Kraszewski and Ormrod (1986) observed larger flower diameter for *Browallia* and *Oxalis,* but smaller floret size for *Crossandra* when plants were grown under high production irradiances. Postproduction longevity was higher when production irradiance was relatively high for begonia (Fjeld, 1986) and *Crossandra* (Kraszewski and Ormrod, 1986).

High production temperatures shorten the forcing period and affect bud development on several azalea cultivars (Pettersen, 1973; Wilkins, 1980). However, we found no information on the effects of forcing temperature on postproduction performance and

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The response of *Leucadendron* 'Safari Sunset' to the fertilization regime

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(*Revised MS received 23 February 2000*)

SUMMARY

The responses of *Leucadendron* 'Safari sunset', on its own roots and grafted on 'Orot' rootstock, to a range of fertilization regimes were studied. The experiment was conducted during the summer of 1994, at Bet Dagan, Israel, and included three levels of compound NPK, two levels of P, and two NH_4:NO_3 ratios.

Ungrafted plants fed without P yielded fewest marketable branches and had the lowest fresh and dry weights. Increasing the P concentration in the irrigation water to 10 mg/dm^3 improved the yield, whereas increasing the NPK level reduced the number of marketable branches and the fresh and dry weight yields. Lowering the NH_4:NO_3 ratio in the irrigation water significantly reduced the yield.

The grafted plants were significantly better than the ungrafted plants under all the treatments examined. The superiority of the grafted plants was more evident under conditions of P deficiency and non-optimal pH.

INTRODUCTION

The Proteaceae family originated in Australia and South Africa, where most species grow on leached, acidic soils that are poor in available minerals. Growth impairment and leaf necrosis or chlorosis are generally attributed to phosphorus toxicity (Nichols *et al.* 1979; Thomas 1980; Buining & Cresswell 1993), and little information is available on the requirements for other nutrients (Parks *et al.* 1996).

An effort has been made in Israel, in the last decade, to cultivate *Leucadendron* 'Safari Sunset' and other Proteaceae (proteas) for the cut flowers market (Ben-Jaacov 1986). 'Safari Sunset' is a clonal selection of an artificial cross between *Leucadendron salignum* (red form) and *L. laureolum* and its great market value and relative ease of cultivation have made it the most important cultivar in the Protea industry. The production of *L.* 'Safari Sunset' is rapidly expanding, and Israeli exports increased from 1·3 million branches in 1992–3 to 24 million in 1997–8. The plant is vigorous, with long, erect branches which terminate in a female cone-like inflorescence, surrounded in the fall by large, deep wine-red bracts.

L. 'Safari Sunset' was bred in New Zealand to suit the local acid soil, and both of its parents are native to soils having low pH. Despite the suitable climate, growers of proteas in Israel have encountered problems because of unfavourable soil characteristics, e.g. high pH and high lime content. To avoid soil limitations, *L.* 'Safari Sunset' is grown in tuff, a volcanic pyroclastic material, characterized by high porosity (0·6 dm^3/dm^3) and high saturated hydraulic conductivity. Silber *et al.* (1998) found that *L.* 'Safari Sunset' plants were not susceptible to P toxicity at normal solution P levels, and that increasing the water-P concentration (up to 20 mg/dm^3) significantly improved plant growth. These findings are in agreement with the conclusions of Prasad & Dennis (1986), that realistic levels of P (below 40 mg P/kg, as measured by bicarbonate extraction) are not toxic to *L.* 'Safari Sunset'.

The common horticultural practice in Israel is to plant *L.* 'Safari Sunset' in tuff inserts embedded in the native soil. Usually, there are no barriers to the free extension of roots from the tuff into the native soil. Thus, roots develop under two different environments: (a) a fixed volume (usually 20–50 dm^3) in the vicinity of the plant, where the tuff properties ensure that the drainage and the pH are suitable for plant growth; and (b) the surrounding native soil, where air deficiency or high pH may restrict plant development.

An alternative approach to avoiding soil constraints is to graft the sensitive plant (scion) onto a soil-adapted rootstock. Some years ago, some species

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native to high-pH soils in South Africa were studied as potential rootstocks for Israeli conditions, and the best-growing variety of *Leucadendron coniferum* was named 'Orot' (Ben-Jaacov *et al.* 1992). 'Orot' is currently widely used as the rootstocks for *L.* 'Safari Sunset'. The objective of the present study was to assess the response to nutritional management of *L.* 'Safari Sunset', on its own roots and grafted onto 'Orot' rootstock.

MATERIALS AND METHODS

The experiment was located at Bet Dagan, Israel (35° E, 31° N, 50 m altitude). The experimental design comprised five treatments, allocated to six completely randomized blocks, with six *L.* 'Safari Sunset' plants – four on their own roots (ungrafted) and two grafted on 'Orot' rootstock (clonal selection of *L. coniferum*) – in each plot. The five treatments (detailed in Table 1) included three compound fertilizer comparisons ($N_1P_1K_1$, $N_2P_2K_2$ and $N_3P_3K_3$), two P treatment comparisons ($N_1P_0K_1$ and $N_1P_1K_1$), and two NH_4:NO_3 treatment comparisons ($N_2P_2K_2$ and NO_3-$N_2P_2K_2$). The nutrient solutions were prepared from commercial fertilizers (NH_4NO_3, $(NH_4)_2SO_4$, KNO_3 and H_3PO_4), and typical Israeli tap water containing (in mg/dm³): N-NO_3 – 10; P – 0·4; K – 6·1; Ca – 50; Mg – 20; Na – 100 and Cl – 140. The pH of the irrigation solutions was 6·8 ± 0·4. Micro-element concentrations (mg/dm³) applied to all the treatments were: Fe – 0·69, Mn – 0·34, Zn – 0·17, Cu – 0·025, Mo – 0·019 and B – 0·25, all EDTA-based, plus 2 mg/dm³ of Fe as EDDHA-Fe.

Two-month-old plants were planted in April 1994, in 40-cm deep holes, dug in the local soil and filled with red tuff (50 litres of 0–8 mm diameter tuff). Some of the surface properties of the tuff are summarized in Table 2 (Silber *et al.* 1994).

Table 1. *Element concentrations in the irrigation water and in leachates from growth containers (average for all seasons)*

Treatment	NH_4-N	NO_3-N	P	K	EC* (dS/m)
	(mg/dm³)				
	Irrigation water				
$N_1P_0K_1$	35	15	0·4	50	1·3
$N_1P_1K_1$	35	15	10	50	1·3
$N_2P_2K_2$	65	35	20	50	1·6
$N_3P_3K_3$	100	50	25	75	1·8
NO_3-$N_2P_2K_2$	35	65	20	50	1·5
	Leachates				
$N_1P_0K_1$	4	60	1·4	70	2·0
$N_1P_1K_1$	4	60	1·8	70	2·4
$N_2P_2K_2$	40	140	3·2	105	3·2
$N_3P_3K_3$	70	160	3·8	145	3·4
NO_3-$N_2P_2K_2$	4	140	2·4	65	2·4

* EC, electrical conductivity.

Table 2. *Surface characteristics of the studied tuff*

Surface characteristic		Comments
Specific surface area	28	N_2 adsorption, m^2/g
pH in H_2O	8·1	90 min shaking, 0·5 g 25/ml
pH in 1 mol/l KCl	6·6	90 min shaking, 0·5 g 25/ml
CEC at pH 7*	285	$mmol_c/kg$

* The tuff has variable charged surfaces contributed mainly from amorphous materials. The relationship between cation exchange capacity (CEC) and pH was found to be: CEC = 41·6 × pH − 6·8.

For each treatment, four control containers filled with 50 litres of tuff, two with and two without plants were installed in the experimental field; they were fertilized identically to the experimental tuff-filled holes and were used to evaluate the net nutrient and water uptakes by the plants. Leachates from all the control containers were collected daily, and their pH, electrical conductivity and volume were monitored. Chemical analyses of the leachates (for N-NH_4, N-NO_3, P, K, Ca, Mg, Fe, Zn and Mn) were performed weekly, where element concentrations were determined as follows: K by flame photometer; reduced N and P by Technicon Autoanalyser; Ca, Mg, Fe, Zn and Mn by atomic absorption spectrophotometer.

The amount of water supplied daily was determined as the highest water uptake level determined in the growth containers, multiplied by 1·2 to account for salt leaching. The leaching factor was raised occasionally, if the electrical conductivity (EC) of the drainage from pots with plants exceeded 3·5 dS/m. Transpiration was calculated from the difference between the volumes of leachates from control containers with and without plants.

To evaluate plant development during the experiment, the trunk diameter was measured five times at a height of 15 cm. At the end of the first season (December 1994), the shoots in each plot were harvested, washed with distilled water, dried at 60 °C in a ventilated oven for 1 week, and pooled for chemical analyses. The dried tissue was ground to pass a 20-mesh sieve, and ashed with H_2SO_4-H_2O_2 for analysis of K, total N and P, or with HNO_3 for Ca and Mg, Fe, Zn and Mn.

Data were subject to analysis of variance (ANOVA) using the GLM procedure (SAS 1985). Separate analyses were performed for the grafted and the ungrafted plants to test the fertilizer effects on each plant type separately. The grafting effect was tested by comparison of the differences of the means of the plant types (grafted and ungrafted) within each replicate plot. Model parameters were fitted by the

NLIN procedure of SAS, using the DUD routine of SAS (SAS 1985).

RESULTS

Yield and plant growth

Ungrafted plants fed without P ($N_1P_0K_1$) yielded the fewest marketable branches (6·9 branches per plant; Table 3), and had the lowest fresh and dry weights (477 and 156 g/plant, respectively).

Increasing the P concentration in the irrigation water up to 10 mg/dm³ ($N_1P_1K_1$) improved the number of marketable branches to 16·5 (ungrafted plants; Table 3) and the fresh and dry weights to 1029 and 330 g/plant, respectively. Phosphorus addition mainly affected the numbers of small and medium branches (40–60 and 60–80 cm, respectively; Table 3). Increasing the compound fertilizer level from $N_1P_1K_1$ to $N_2P_2K_2$ did not improve plant yield (Table 3) but significantly decreased the number of long branches (80 cm; Table 3). A further increase in fertilizer (to $N_3P_3K_3$) reduced the number of marketable branches and the fresh and dry weights (Table 3). Lowering the NH_4:NO_3 ratio in the irrigation water (NO_3-$N_2P_2K_2$ compared with $N_2P_2K_2$) significantly reduced the number of marketable branches and the fresh and dry weights (Table 3). The leaves of NO_3-$N_2P_2K_2$-fed plants were small and their stem elongation was inhibited, with a "rosette" like appearance. For each fertilizer treatment, grafted plants produced higher yields than ungrafted plants (Table 3).

Root exposure at the end of the first year (December) demonstrated that at least 80% of the root system was located in the tuff. The root system in

Table 3. *Treatment effects on the total number of marketable branches (Br.), fresh and dry weights (FW and DW, respectively) and length distribution of ungrafted* L. *'Safari Sunset' and grafted ('Orot') plants*

	Br. (No. per plant)		FW (g per plant)		DW (g per plant)	
Shoot Treatment	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'
$N_1P_0K_1$	6·9	15·0	477	922	156	305
$N_1P_1K_1$	16·5	20·8	1029	1213	330	389
$N_2P_2K_2$	15·0	20·0	1066	1156	328	383
$N_3P_3K_3$	9·1	20·5	822	992	258	328
NO_3-$N_2P_2K_2$	7·2	19·8	738	1237	203	399
Mean	10·9	19·2	826	1104	255	361
Fertilizers	**	NS	**	NS	**	NS
5% LSD (D.F. = 20)†	5·69	8·31	283·9	456·6	99·8	155·1
Grafting	***		**		***	
5% LSD (D.F. = 25)‡	3·06		167·4		57·4	
Fert. × Graft.	NS		NS		NS	

	Marketable shoot lengths (No. per plant)					
	40–60 cm		60–80 cm		> 80 cm	
Shoot Treatment	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'
$N_1P_0K_1$	3·5	5·8	1·6	3·7	1·8	4·8
$N_1P_1K_1$	11·0	9·7	4·0	7·7	1·5	4·3
$N_2P_2K_2$	11·7	16·0	2·9	3·3	0·4	2·0
$N_3P_3K_3$	7·9	13·0	1·0	4·5	0·1	5·8
NO_3-$N_2P_2K_2$	4·5	9·3	1·7	5·5	0·5	5·0
Mean	7·8	10·8	2·3	4·9	0·8	4·4
Fertilizers	**	*	*	*	**	NS
5% LSD (D.F. = 20)†	4·82	6·50	2·14	2·64	1·01	7·30
Grafting	**		***		***	
5% LSD (D.F. = 25)‡	2·63		1·55		1·62	
Fert. × Graft.	NS		NS		NS	

*, **, ***, significant at $P \leq 0{\cdot}05$, 0·01 and 0·001, respectively.
NS, not significant.
† LSD for comparing any pair of fertilizer treatments within grafting treatments.
‡ LSD for comparing the grafted versus ungrafted treatment means.

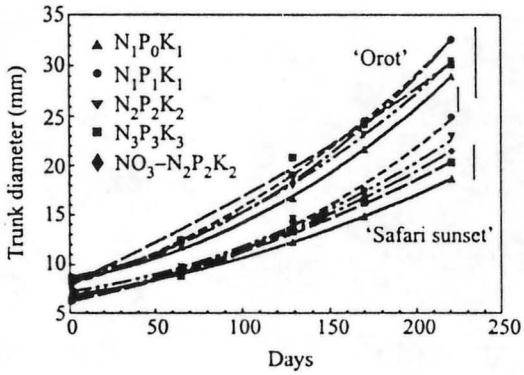

Fig. 1. Trunk diameter (mm) of *L.* 'Safari Sunset' grown on its own roots and on 'Orot' rootstock, as a function of time (days, day 0 was 1 May 1994). The regression coefficients are presented in Table 4. Vertical lines at the right indicate LSD ($P \leqslant 0.05$) of trunk diameter measured at harvest: bottom – for 'Safari Sunset'; middle – for the means of grafted versus ungrafted plants; top – for grafted plants on 'Orot'.

the tuff was healthy and white with good branching, whereas in the soil the root system was restricted and brown with poor branching. The development and yields of plants in the growth container were identical to those of plants grown in the field under the same treatment.

Trunk diameter

The trunk diameter increased during the experiment in accordance with the nutritional treatments (Fig. 1). The trunk diameter (D_i; mm) was described by a second-degree polynomial:

$$D_i = a_i + b_i \times t + c_i \times t^2 \qquad [1]$$

where a_i, b_i and c_i represent empirical constants (detailed in Table 4), t is time in days and the subscript i indicates the treatment.

A significant linear regression was obtained between the trunk diameter and the fresh and dry weights of the plants at the end of the experiment ($R^2 = 0.91$ and 0.80, respectively, not presented), indicating that the trunk diameter can serve as an indestructible indicator of plant development during the growth period.

Water uptake

The tuff contained the major and essential active part of the root system, therefore, the transpiration could be reliably estimated according to the data on leachate from the control containers. As only ungrafted plants were planted in the control containers, water uptake by grafted plants could not be estimated. Daily transpiration increased from 0·1 dm^3/plant at the beginning of May to more than 4 dm^3/plant at the beginning of October (Fig. 2).

If we assume that the amount of water needed for leaching salt from the root area is about 20% of the water added, and that the daily evaporation from tuff is ~ 1 dm^3/plant, then the maximum amount of water needed for ungrafted *L.* 'Safari Sunset' during the first year is about 6 dm^3/day. The common practice in Israel is to plant 700 plants per 1000 m^2, hence the daily rate for *L.* 'Safari Sunset' plants is 4·2 mm; this may be compared with average daily pan evaporation rates of 6·2, 4·8 and 3·2 mm, for September, October and November, respectively, near the experimental site. The transpiration by plants fed with a low NH_4:NO_3 ratio (NO_3-$N_2P_2K_2$) was lower than that of $N_2P_2K_2$ plants, which is consistent with their lower dry weight production (Fig. 2, Table 3).

Table 4. *Regression coefficients describing the effect of time on the trunk diameter (Eqn. [1]) of* L. *'Safari Sunset' and 'Orot'. The standard errors of the coefficients are shown in parentheses*

	Treatment*		
	a	b	c
'Safari Sunset'			
$N_1P_0K_1$	6·9 (0·02)	0·02 (3.9×10^{-4})	1.3×10^{-4} (1.7×10^{-7})
$N_1P_1K_1$	6·5 (0·29)	0·04 (6.0×10^{-3})	1.5×10^{-4} (2.6×10^{-5})
$N_2P_2K_2$	7·4 (0·96)	0·02 (2.1×10^{-3})	2.4×10^{-4} (1.8×10^{-5})
$N_3P_3K_3$	6·2 (0·22)	0·03 (4.5×10^{-3})	1.4×10^{-4} (2.0×10^{-5})
NO_3-$N_2P_2K_2$	6·6 (0·30)	0·03 (5.2×10^{-3})	1.5×10^{-4} (2.7×10^{-5})
'Orot'			
$N_1P_0K_1$	8·6 (0·23)	0·03 (4.7×10^{-3})	3.0×10^{-4} (2.1×10^{-5})
$N_1P_1K_1$	8·0 (0·04)	0·05 (8.0×10^{-4})	2.7×10^{-4} (3.4×10^{-6})
$N_2P_2K_2$	8·1 (0·27)	0·04 (5.7×10^{-3})	2.5×10^{-4} (2.5×10^{-5})
$N_3P_3K_3$	8·2 (1·02)	0·07 (2.3×10^{-3})	1.1×10^{-4} (1.0×10^{-5})
NO_3-$N_2P_2K_2$	8·8 (0·18)	0·03 (3.8×10^{-3})	3.6×10^{-4} (1.7×10^{-5})

* a, b, c represent empirical constants (see text above).

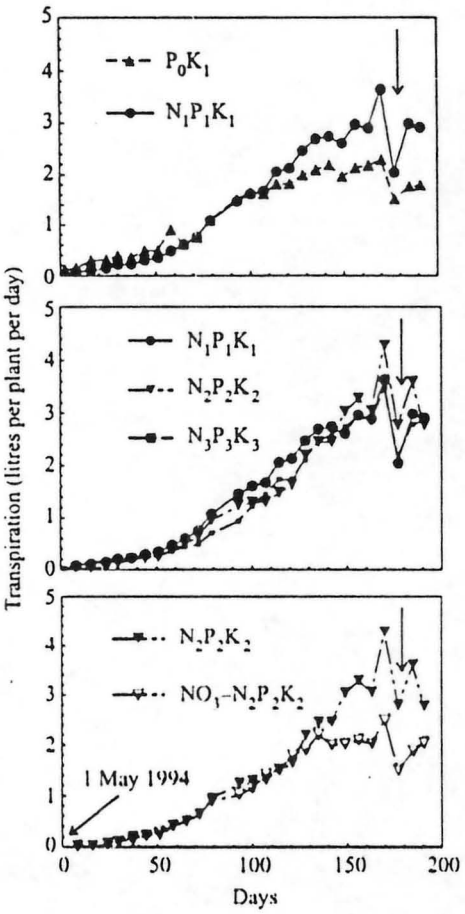

Fig. 2. Treatment effects on the daily transpiration rate of ungrafted plants grown in control containers. Arrows indicate rain.

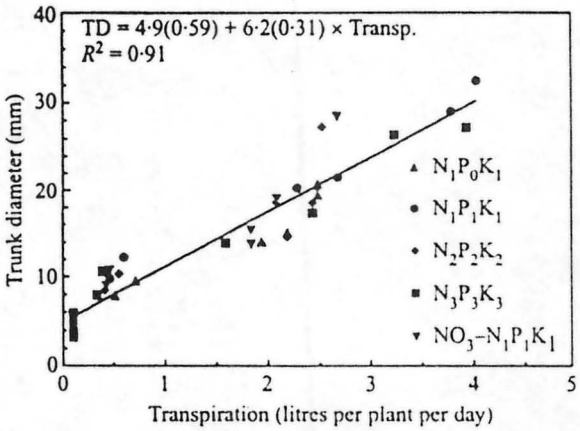

Fig. 3. Trunk diameter (TD; mm) of ungrafted plants as a function of the daily transpiration rate. The standard error of each parameter is presented in brackets.

The transpiration value predicted for the production of 1 kg dry matter of zero-P-fed plants was 1282 dm^3, compared with 450 ± 30 dm^3 for $N_1P_1K_1$, $N_2P_2K_2$ or $N_3P_3K_3$ fed plants, and 520 dm^3 for NO_3-$N_2P_2K_2$ plants. The low water use efficiency of the NO_3-$N_2P_2K_2$ and especially, the zero-P-fed plants indicates the influence of limiting factors such as high pH or P deficiency, respectively (see below).

A significant linear regression was obtained between trunk diameter and the water uptake by plants in the growth containers (Fig. 3). Assuming the same linear relationship for the grafted plants, the estimated peak water consumption by a grafted plant is more than 5 dm^3 per day, and the daily amount of water needed for irrigation is about 5·5 mm.

Element concentrations in plants

The N and P concentrations in the shoots of both ungrafted and grafted zero-P-fed plants were significantly lower than those in other treatments (Table 5).

The addition of P to the irrigation water resulted in increases in both N and P concentrations in the shoot tissue indicating that P deficiency probably restricted N uptake by the plants. Raising the NPK compound fertilizer level from $N_1P_1K_1$ to $N_2P_2K_2$ increased the N and P concentrations in the shoots, but this effect was significant only for the ungrafted plants (Table 5). However, the actual increase was similar for both plant types and the lack of significant effect for the grafted plants was probably due to the fewer number of grafted plants. Phosphorus concentrations in the shoots of both ungrafted and grafted plants increased significantly as a result of reduction of the NH_4:NO_3 ratio (NO_3-$N_2P_2K_2$ compared with $N_2P_2K_2$, Table 5). This finding is consistent with the finding of a previous experiment on *L.* 'Safari Sunset' grown in an aero-hydroponic system (Silber *et al.* 1999*a*) but conflicts with the behaviour expected according to the anion–cation balance mechanism (Mengel & Kirkby 1987). The P concentration in the ungrafted plants was at a level that can be regarded as normal for most plants, whereas that in the grafted plants was low (Marschner 1995).

The K concentration in shoots of both ungrafted and grafted *L.* 'Safari Sunset' plants was very low (Table 5) compared with those reported for other ornamental plants. The decrease in K concentration as a result of P addition ($N_1P_1K_1$ compared with $N_1P_0K_1$ treatments, Table 5) was probably a dilution effect. The low K requirement of the proteaceous plants may be attributed to an adaptation to the low-K soils on which they originated, as suggested previously by Walters *et al.* (1991) and Parks *et al.* (1996).

Calcium, Mg, Fe, Zn and Mn concentrations in the leaves of plants from all treatments except NO_3-$N_2P_2K_2$ were unaffected by the fertilizer application, therefore only the comparisons between $N_2P_2K_2$ and

Table 5. *Treatment effects on element concentrations (g/kg DW) in shoots of* L. '*Safari Sunset*' *and grafted* ('*Orot*') *plants*

Shoot Treatment	N		P		K	
	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'	'Safari Sunset'	'Orot'
$N_1P_0K_1$	12·5	13·2	0·4	0·5	6·8	5·0
$N_1P_1K_1$	14·7	16·2	3·5	1·3	5·6	3·9
$N_2P_2K_2$	16·2	18·0	5·2	1·5	6·1	4·2
$N_3P_3K_3$	16·9	18·2	6·1	2·3	7·5	4·4
NO_3-$N_2P_2K_2$	14·3	16·5	6·2	2·6	7·0	3·7
Mean	14·9	16·4	4·3	1·6	6·6	4·3
Fertilizers	***	**	***	***	*	*
5% LSD (D.F. = 20)	1·26	2·39	0·93	0·68	1·17	0·86
Grafting	***		***		***	
5% LSD (D.F. = 25)	0·82		0·35		0·45	
Fert. × Graft.	NS		***		NS	

All symbols and abbreviations as in Table 3.

Table 6. *Effect of* NH_4:NO_3 *ratio on cation concentrations in shoots of* L. '*Safari Sunset*' *(average of ungrafted and grafted) plants*

Treatment	Ca (g/kg DW)	Mg (g/kg DW)	Fe (mg/kg DW)	Zn (mg/kg DW)	Mn (mg/kg DW)
$N_2P_2K_2$	13·6 (S.E. = 0·14)	6·8 (S.E. = 0·66)	250 (S.E. = 20·2)	117 (S.E. = 15·8)	280 (S.E. = 40·2)
NO_3-$N_2P_2K_2$	8·8 (S.E. = 0·92)	5·2 (S.E. = 0·89)	127 (S.E. = 5·7)	51 (S.E. = 3·6)	111 (S.E. = 10·1)

NO_3-$N_2P_2K_2$ are shown in Table 6. There were no significant grafting effects; therefore the means of the grafted and ungrafted plants are shown.

In contrast with previous research results (Marschner 1995), cation concentrations in NO_3-N-fed plants were found to be lower than those in NH_4-N-fed plants (Table 6). The decrease in cation concentrations which resulted from the reduced NH_4:NO_3 ratio conflicts with the result of a previous experiment on *L.* 'Safari Sunset' grown in an aero-hydroponic system (Silber *et al.* 1999*a*) and may be a result of a secondary effect of the NH_4:NO_3 ratio on pH (see below).

pH in the leachates

The rhizosphere pH was evaluated from the pH of the leachates from the growth containers. Increased nutrient levels made more ammonium ions (Table 1) available for exchange and nitrification reactions and, consequently, more protons were released and the pH declined ($N_3P_3K_3$ versus $N_2P_2K_2$ and $N_1P_1K_1$; Fig. 4).

Conversely, lowering the NH_4:NO_3 ratio increased the pH (NO_3-$N_2P_2K_2$ versus $N_2P_2K_2$; Fig. 4). Phosphorus addition did not affect the pH ($N_1P_1K_1$ versus $N_1P_0K_1$; Fig. 4).

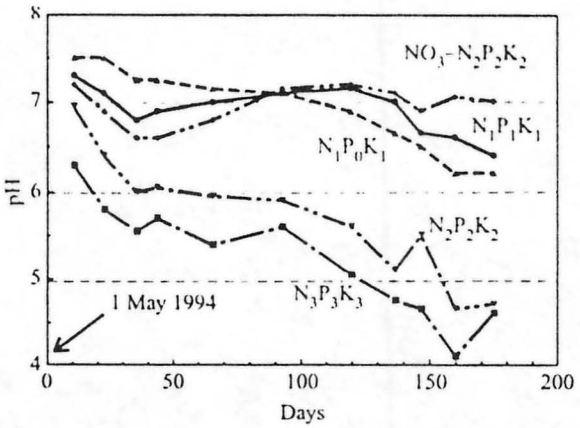

Fig. 4. pH in leachates from growth containers with plants, as functions of time and the treatments applied.

Element concentrations in the leachates

Nitrate, total inorganic-N and K concentrations in leachates (control containers with plants) were higher than in the irrigation water (Table 1), indicating that N and K concentration were not limiting factors in

the experiment. Total-N and K concentrations increased because of preferential water uptake. At low NH_4^+ concentrations (35 mg/dm^3), adsorption on the solid phase reduced NH_4^+, whereas nitrification increased NO_3^- concentrations. Ammonium-ion concentrations in leachates increased up to 70 mg/dm^3, as the NH_4^+ concentration in the irrigation water was increased to 65 and 100 mg/dm^3. The increased NH_4^+ concentrations resulted from one or more of three possible mechanisms: (a) a saturation effect that reduced the oxidation rate of NH_4^+; (b) diminished NH_4^+ adsorption on the solid phase as a result of the diminution of tuff cation exchange capacity (CEC) caused by lowering the pH (Table 1); and (c) impairment of micro-organism activity by lowered pH leading, in turn, to reduced nitrification. The pH effects (mechanisms (b) and (c)) are probably more important than the saturation effect (mechanism (a)).

Adsorption and precipitation reactions reduced P concentrations in the tuff medium to approximately 10% of that in the irrigation water (Table 2). The higher P concentration in leachates of zero-P-fed plants was a result of indigenous P release from the tuff (Table 2).

DISCUSSION

Probably the two main factors that controlled the ungrafted plant development in the present experiment were: (a) the direct effect of P application; and (b) the indirect effect of the pH in the rhizosphere. The levels of P and NH_4^+ increased simultaneously in the $N_1P_1K_1$, $N_2P_2K_2$ and $N_3P_3K_3$ treatments, therefore, distinguishing between the two effects is not possible.

A significant quadratic regression was obtained between the number of marketable branches and the P concentration in the leaves (a) and in the irrigation water (b) of the ungrafted NH_4-N-fed plants. There were similar relationships for the fresh and dry weights of shoots (not presented). No corresponding significant relationships were obtained for the grafted plants.

The NO_3-$N_2P_2K_2$ treatment was omitted from the above regression, since changing the NH_4:NO_3 ratio significantly altered the rhizosphere pH (Fig. 4). The quadratic regression function in Fig. 5*b*, shows that the maximum number of marketable branches on ungrafted plants was achieved when the P concentration in the irrigation water approached 13–14 mg/dm^3. Phosphorus application affected P uptake by ungrafted plants (Table 5), leading to the observed significant regression relationship between the number of marketable branches, the fresh and the dry weights of shoots, and the P concentration in the leaves. The quadratic regression function in Fig. 5*a* shows that the leaf-P concentration required for maximum yield was 3·4 g/kg DW. The above P concentrations are typical of many families of plants (Marschner 1995),

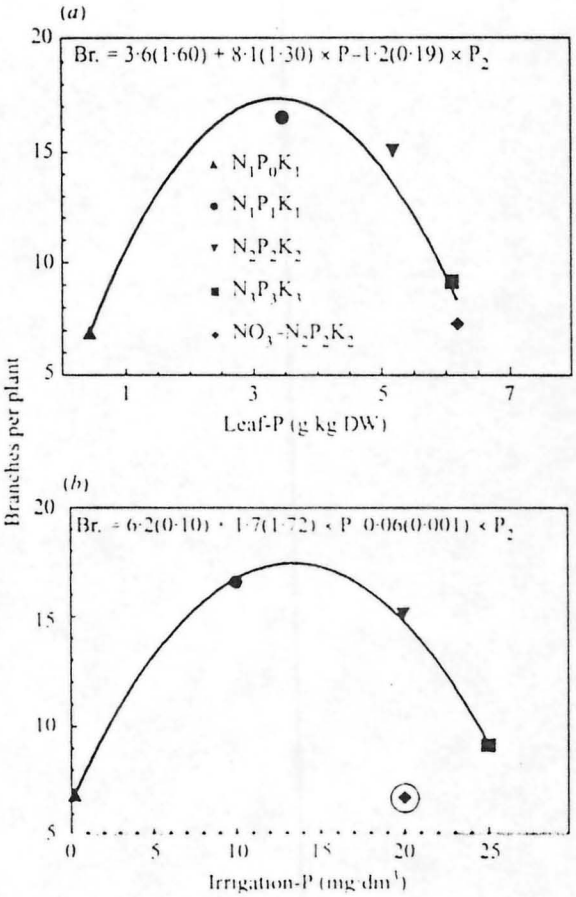

Fig. 5. Number of marketable branches (Br.) per plant (ungrafted), as quadratic functions of: (*a*) the leaf-P (g/kg DW), and (*b*) the irrigation-P concentration (mg/dm^3). The standard error of each parameter is presented in brackets. The NO_3-$N_2P_2K_2$ treatment (encircled in the lower figure) was omitted from the irrigation-P regression because changing the NH_4:NO_3 ratio altered the rhizosphere pH.

indicating that *Leucadendron* plants are not susceptible to P toxicity at normal P application rates.

The pH in the rhizosphere is probably an important factor in the growth of ungrafted *L.* 'Safari Sunset'. Omitting the $N_1P_0K_1$ treatment, where the direct effect of P deficiency clearly restricted plant development, the relationship between the yield and the average leachate pH during the main growth season (100–200 days) gave an estimate of the optimum pH needed for maximum plant growth. Based on quadratic regression (not presented) the maximum number of marketable branches was achieved when the pH in leachates from containers with plants was 6·0. Below this value, release of toxic Mn and Al from tuff constituents (Silber *et al.* 1999*b*) have impaired plant development. Furthermore, since it can be assumed that the actual pH in the vicinity of the roots would

be more acid than the data in Fig. 4, the risk of impairing plant development by lowering the feed pH below 6·0 could be substantial.

At pH 7·0 and above, despite the use of chelates, the availability of micronutrients was probably too low to provide adequate uptake. Iron, Zn and Mn concentrations in shoots of NO_3-N-fed plants were lower than those in all NH_4-N-fed plants (Table 6) which indicates that inadequate metal uptake may have limited the development of NO_3-N-fed plants. If the pH in the vicinity of the roots was assumed to have been even higher than the value measured in the NO_3-$N_2P_2K_2$ leachates (Fig. 4), the low availability of metal ions would have been underestimated. The "little leaf" and the "rosette" appearance of NO_3-$N_2P_2K_2$-fed plants as well as the higher P concentrations in their leaves (Table 5), could have been a result of Zn deficiency. Zinc deficiency could have enhanced P uptake in shoots (Loneragan & Webb 1993; Marschner 1995) due to: (a) enhanced permeability of the plasma membranes of root cells; (b) impairment of the mechanism which controls the loading of P into the xylem; or (c) specific impairment of the retranslocation of P in the phloem.

CONCLUSIONS

L. 'Safari Sunset' plants are not susceptible to P toxicity at normal P application rates. The growth of grafted plants was significantly superior to that of ungrafted plants under all the treatments examined. The advantage of the grafted plants was more significant under conditions of P deficiency and non-optimal pH. The rhizosphere pH is a very important factor affecting *L.* 'Safari Sunset' growth. Whether pH affects the plants directly through physiological mechanisms or indirectly through its effects on nutrient availability is not clear. Micronutrient concentrations in leaves of plants grown at pH above 7 were lower than those in plants grown at lower pH, which seems to indicate that inadequate metal acquisition limited the growth of these plants.

REFERENCES

Ben-Jaacov, J. (1986). Protea production in Israel. *Acta Horticulturæ* **195**, 101–110.

Ben-Jaacov, J., Ackerman, A., Gilad, S., Carmeli, R., Barzilay, A & Schori, Y. (1992). Grafting techniques and the use of rootstocks in *Leucadendron*, and other Proteaceous plants. *Acta Horticulturæ* **316**, 69–71.

Buining, F. & Cresswell, G. (1993). Working party on nutrition of Proteaceae. *International Protea Association* **26**, 21–27.

Loneragan, J. F. & Webb, M. J. (1993). Interactions between zinc and other nutrients affecting the growth of plants. In *Zinc in Soils and Plants* (Ed. A. D. Robson), pp. 119–134. Dordrecht: Kluwer Academic Publishers.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. *2nd edn*. San Diego: Academic Press Limited.

Mengel, K. & Kirkby, E. A. (1987). *Principles of Plant Nutrition*. *4th edn*. Bern: International Potash Institute.

Nichols, D. G., Jones, D. L. & Beardsell, D. V. (1979). The effect of phosphorus on the growth of *Grevillea* 'Poorinda Firebird' in soil-less potting- mixtures. *Scientia Horticulturæ* **11**, 197–206.

Parks, S. E., Cresswell, G. C., Haigh, A., Buining, F. & Barlow, E. W. R. (1996). Nutritional requirements of some Proteaceous plants. In *Proceeding of the IV National Workshop for Australian Native Flowers*, pp. 210–215. Perth, Australia.

Prasad, L. & Dennis, D. J. (1986). Phosphorus nutrition of *Leucadendron* Safari Sunset. *Acta Horticulturæ* **185**, 155–162.

SAS (1985). *SAS User's Guide*. Cary, NC: SAS Inst. Inc.

Silber, A., Bar-Yosef, B., Singer, A. & Chen, Y. (1994). Mineralogical and chemical composition of three tuffs from northern Israel. *Geoderma* **63**, 123–144.

Silber, A., Ganmore-Neumann, R. & Ben-Jaacov, J. (1998). Effects of nutrient addition on growth and rhizosphere pH of *Leucadendron* 'Safari sunset'. *Plant and Soil* **199**, 205–211.

Silber, A., Ganmore-Neumann, R., Ackerman, A., Mitchnick, B. & Ben-Jaacov, J. (1999*a*). pH dominates *Leucadendron* 'Safari sunset' development. *Hortscience* (in press).

Silber, A., Bar-Yosef, B. & Chen, Y. (1999*b*). pH dependent kinetics of tuff dissolution. *Geoderma* **93**, 125–140.

Thomas, M. B. (1980). Phosphorus response of Proteaceae and other nursery plants in containers. *Annual Journal of the Royal New Zealand Institute of Horticulture* **8**, 21–33.

Walters, C. M., Jooste, J. H. & Raitt, L. N. (1991). Aspects of the sodium and potassium nutrition of the fynbos shrub *Leucadendron salignum* L. (Proteaceae). *South African Journal of Botany* **57(4)**, 181–185.

Leaf chemical composition and nutrient removal by stems of *Leucadendron* cvv. Silvan Red and Safari Sunset

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Summary. Chemical analysis was used to determine the concentrations of 12 nutrients in youngest fully expanded leaves (YFEL) of *Leucadendron* cvv. Silvan Red and Safari Sunset at 2 sites in the Mount Lofty Ranges. Leaves were sampled every month for 3 years, commencing in July 1990. The leaf nutrient data were used to define seasonal nutrient trends, thereby identifying the most suitable time for leaf analysis; to determine the magnitude of the differences in leaf nutrient composition between *Leucadendron* cultivars, and between *Leucadendron* and *Protea* hybrids; to calculate total nutrient removal by harvested stems, which can be used to formulate maintenance fertiliser programs; and to determine the correlations between nutrients.

The seasonal increase in concentrations of nitrogen (N), phosphorus (P), potassium (K), and sodium (Na) in YFEL corresponded with the spring growth flush, after which concentrations decreased with time, particularly during summer and autumn. Concentrations of copper (Cu) and zinc (Zn) were unstable during October–April and the seasonal trends were not consistent between sites or with other mobile nutrients (e.g. N, P, K). Concentrations of calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), sulfur (S), and boron (B) at site 1 decreased early in the season, were lowest when vegetative flushing peaked, and tended to increase during autumn and winter.

Seasonal variation in the main nutrients removed in marketable stems (i.e. N, Ca, K, Mg) was minimal during June–August. However, to assess the overall nutrient status of plantings, sampling in June is most suitable. Crop nutrient surveys conducted at this time, in conjunction with productivity and quality data, can be used to develop interpretation standards for leaf analysis.

For all nutrients, the seasonal trends were similar for the 2 cultivars, but concentrations of Mn were consistently lower in YFEL of Silvan Red than Safari Sunset. In contrast to the small differences between cultivars, there were large differences in leaf nutrient composition between the *Leucadendron* cultivars and *Protea* 'Pink Ice'. For example, Mg, Na, and Mn concentrations were consistently lower, and N, K, Ca, and Fe higher, in YFEL of Pink Ice than in the *Leucadendron* cultivars. For these nutrients, different interpretation standards may be required for *Leucadendron* and *Protea* hybrids.

The major nutrients removed in harvested stems were Na, N, Ca, K, and Mg. Based on nutrient uptake data alone, we suggest annual applications of N and Ca at 20–30 g/plant, and Mg and K at 10–15 g/plant, on acid sands.

Significant ($P<0.05$) correlations were found between many nutrients. For example, N concentrations were positively correlated with P, K, Na, and Zn, and negatively correlated with Ca, Mg, and Fe concentrations. These significant relationships may indicate synergistic and antagonistic interactions between nutrients, which need to be considered when interpreting plant test data.

Introduction

Commercial plantings of species of the family *Proteaceae* have increased significantly in Australia over the past 15 years. Among these species, *Leucadendron* are popular for their colourful floral foliage (involucral leaves) and long harvest period. Cultivars Silvan Red and Safari Sunset, which are *Leucadendron laureolum* x *L. salignum* hybrids, are widely grown throughout Australia. Many plantings are now 5–10 years old and little is known about their nutrient or fertiliser requirements. Although many *Proteaceae* species are reported to utilise soil nutrients efficiently and therefore have a low nutrient requirement (Claassens 1986; Parvin 1986), continual removal of large amounts of biomass each year as marketable stems and prunings may gradually deplete

soil nutrient reserves. Growers in Australia have no calibrated plant or soil tests to assess the nutrient status of their *Leucadendron* plantings. Such tests would allow objective nutrient management decisions. The calibration of a plant test is also important because nutrient deficiency symptoms may be confused with symptoms of disease or other forms of stress. Further, once symptoms are obvious, plant growth and stem quality may already be adversely affected. Using leaf colour symptoms to diagnose nutrient stress in *Leucadendron* species is also confounded by the normal changes in foliage colour that occur during the year.

Few leaf nutrient data have been published for *Leucadendron* species. In Australia, Cresswell (1991) studied the effects of increasing rates of phosphorus (P) on growth and the concentration of P in different tissues of *Leucadendron* cv. Harvest grown in pots. Heinsohn and Parmenter (1986), working in South Africa, studied nitrogen (N), P, and potassium (K) nutrition of *Leucadendron salignum* seedlings in water culture. We do not know of any published data on the leaf chemical composition of commercial *Leucadendron* plantings in Australia. By monitoring the nutrient status of commercial plantings with desired productivity and quality characteristics, interpretation standards for diagnostic plant testing can be developed. Many factors must be considered to ensure that reliable diagnostic standards are developed (Cresswell 1989; Lewis *et al.* 1993). An example is seasonal trends in leaf nutrient composition, which have been reported for many horticultural crops (Maier *et al.* 1995). With knowledge of the magnitude of these trends, the preferred sampling time for plant analysis (i.e. when the rate of change in nutrient concentration is minimal) can be determined. We therefore monitored the seasonal trends in nutrient composition of youngest fully expanded leaves (YFEL) of *Leucadendron* cvv. Silvan Red and Safari Sunset at commercial plantings in South Australia for 3 years. These trends were used to identify the preferred sampling time for leaf analysis. Surveys of the nutrient status of commercial plantings can then be conducted, and the leaf nutrient data, in conjunction with productivity and quality information, can be used to develop interpretation standards (Cresswell 1989). We also compared the nutrient concentrations of the 2 *Leucadendron* cultivars with those for *Protea* 'Pink Ice' (Maier *et al.* 1995), to identify differences between species.

When decisions on crop nutrient requirements are based on leaf analysis data, relationships between nutrients within the plant must be considered. For example, in potatoes, the synergism between total N and P, and the negative relationship between nitrate-N and chloride (Cl^-), will affect interpretation of petiole analysis data (Maier *et al.* 1994). Similarly with perennial crops, Sanchez-Alonso and Lachica (1987*a*, 1987*b*) reported highly significant correlations (r values up to 0.95) between nutrients in leaves of plum and cherry. They concluded that defining such relationships may be useful for making decisions on fertiliser requirements. We therefore determined the correlations between nutrients in YFEL of Silvan Red and Safari Sunset.

A calibrated plant test cannot predict the amount of fertiliser required when low concentrations or deficiencies are diagnosed. The 'balance sheet' approach (Lewis 1985; Prance 1992; Sparrow 1993) is one way to determine amounts of nutrients to apply. Knowledge is required of the amounts of nutrients removed in produce. We determined the nutrient composition of whole stems of *Leucadendron* spp. at harvest to estimate the amounts of nutrients removed by stems.

Materials and methods

Sites

This study was carried out over 3 years on 2 well-managed commercial plantings in the Mount Lofty Ranges. The plants were healthy and showed no symptoms of nutrient stress. The cultivars studied were Silvan Red and Safari Sunset at site 1, and Silvan Red at site 2. For Silvan Red, productivity and growth were assessed (to be presented separately); total yields of stems harvested from 6 plants in each planting are presented to indicate the productivity of the plantings. At site 1, the mean (± s.e.) total numbers of stems harvested were 289 ± 22, 330 ± 11, and 324 ± 26 per plant in 1991, 1992, and 1993, respectively. For site 2, the numbers were 222 ± 32, 209 ± 26, and 226 ± 5, respectively. No yield data have been published in Australia with which to compare these results; however, the yields at these 2 sites compare favourably with those at another site, where the plants yielded only 81 and 80 stems/plant in 1992 and 1993, respectively (N. A. Maier unpublished data).

The climate is classified as Mediterranean, with cool, wet winters and dry, warm summers. Pest and disease control, irrigation, and fertiliser management were carried out by the grower. Nutrient management of the plantings is discussed in more detail below in relation to soil test data. Plants at all sites were drip-irrigated. When monitoring commenced in July 1990, the plants were 5 years old at site 1 and 6 years old at site 2. Soil types were loamy sand or clay loam over clay (site 1, Safari Sunset); loamy sand or sandy loam over clay (site 1, Silvan Red); and sand over sandy loam (site 2). Selected chemical and physical properties of the soil at each site are presented in Table 1.

Leaf sampling procedure

To determine the magnitude of seasonal nutrient trends, leaf sampling was carried out monthly at each site from July 1990 to June 1993. At each sampling, at

Table 1. Chemical and physical properties of the soil at two sites in the Mount Lofty Ranges

Extractable P and K, 1 : 100 soil : 0.5 mmol $NaHCO_3$/L, 16 h shaking time (Colwell procedure); extractable Cu, Zn, Mn, and Fe, DTPA extraction

Depth (cm)	pH[A]	EC[A] (mS/cm)	P	K	Cu	Zn	Mn	Fe	Organic C[B]	Sand	Silt	Clay
					(mg/kg)					(%)		
Site 1, Safari Sunset planting												
0–10	5.2	0.09	32	360	0.6	5.9	28.0	45.0	5.5	78	14	8
>10–20	5.3	0.06	23	360	0.3	0.9	2.0	41.0	1.9	49	18	33
>20–40	5.5	0.04	7	350	0.2	0.6	0.3	12.0	1.1	27	15	58
>40–60	5.6	0.03	4	350	0.1	0.1	0.0	2.0	0.5	18	11	71
Site 1, Silvan Red planting												
0–20	5.2	0.03	16	123	0.7	2.6	9.4	45.0	3.5	86	9	5
>20–30	5.0	0.03	18	95	0.3	0.7	0.9	45.0	1.0	81	10	9
>30–45	5.4	0.03	13	92	0.3	0.5	1.0	45.0	0.8	82	9	9
>45–60	5.7	0.03	4	107	0.1	0.2	0.2	11.6	0.4	48	14	39
Site 2, Silvan Red planting												
0–20	7.0	0.03	14	43	0.3	1.1	0.9	8.8	0.4	95	2	3
>20–70	6.8	0.03	9	48	0.1	0.3	0.1	10.7	0.1	90	4	6
>70–1000	6.9	0.05	4	60	0.1	0.9	0.2	7.9	0.1	87	4	9

[A] 1 : 5 soil : water (w/v).
[B] Walkley and Black (1934).

least 50 leaves were collected from healthy plants throughout the planting. The index tissue sampled was the youngest fully expanded leaf (YFEL) from a shoot >40 cm long without a visible flower.

Nutrient composition and removal in harvested stems

To estimate nutrient composition and removal by harvested stems, fresh and dry weights of individual stems sampled at harvest were determined before chemical analysis. Over the 3 years of the study, the total number of stems sampled was 6 from Safari Sunset and 7 from Silvan Red at site 1, and 14 from Silvan Red at site 2. To calculate nutrient removal, we used a total stem yield of 8 kg/plant, fresh weight. Based on our harvest data, this is a reasonable yield per plant from a productive planting of these 2 cultivars. Similar calculations were made by Maier *et al.* (1995) for Pink Ice, using yield of 50 stems/plant, and by George *et al.* (1989) for custard apple, using 100 kg of fruit.

Soil sampling

To characterise the soil, samples were collected at each site in May 1992. Soil profiles were studied using a 7.5-cm auger at up to 3 locations at each planting. The depth of different soil horizons was determined by noting changes in texture or colour. Data are presented for 1 representative location at each site.

Analytical procedure

All leaf and stem samples were dried at 60–70°C in a forced-draught oven and ground to <1 mm before analysis. The samples were analysed for total N, P, K, calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), boron (B), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe), using procedures described by Maier *et al.* (1995).

All soil samples were air-dried and ground to <2 mm before chemical analysis (Table 1; Maier *et al.* 1995).

Statistical methods

To show the seasonal trends, mean monthly nutrient concentrations for each site are presented graphically. Means and standard errors were calculated using 3 years data (n = 3). Representative standard errors are presented on the graphs to indicate variability between years. To determine the differences in nutrient concentrations and seasonal trends for the 2 *Leucadendron* cultivas and Pink Ice, we visually compared the graphs. For those nutrients where there were consistent differences, we calculated the mean, (± s.e.) and range of the monthly values presented in the graphs (n = 12), to illustrate the magnitude of the difference. For the comparisons, we only used the data collected at site 1 to minimise site effects. The graphs showing the seasonal trends of nutrient concentrations in YFEL of Pink Ice were presented in Maier *et al.* (1995). The planting referred to as site 1 in the Pink Ice study was also used in the leucadendron work (site 1). Mean (± s.e.m.) nutrient concentrations and nutrient removal in harvested stems were calculated for Silvan Red at sites 1 (n = 7) and 2 (n = 14) and for Safari Sunset at site 1 (n = 6). Correlation coefficients (r) for linear relationships between all nutrients in YFEL were determined. Only r values significant at P = 0.05 are presented.

Results and discussion

Soil chemical and physical properties

Soil pH was near neutral throughout the profile at site 2, but strongly acid to 40–45 cm at site 1 (Table 1). Although the soil at site 1 was strongly acid, the yield data presented above showed that the productivity of this Silvan Red planting was high compared with others. We therefore suggest that these *Leucadendron* cultivars are tolerant of strongly acid soils of pH 5–5.5. Consistent with this hypothesis, N. A. Maier (unpublished data) found that for Safari Sunset growing in similar soil types, increasing the annual rate of applied N (as ammonium sulfate) from nil to 25 g N/plant significantly ($P<0.05$) decreased soil pH from 5.0 to 4.4 at one site, and from 5.4 to 4.9 at another. The productivity of the plants was not significantly ($P>0.05$) decreased during the course of the experiments. Maier *et al.* (1995) also suggested that Pink Ice was tolerant of pH values in the range 5.5–4.8.

Based on data for the 2 sites, extractable P and K concentrations in the surface (0–20 cm) soils ranged from 14 to 32 mg/kg and 43 to 360 mg/kg, respectively (Table 1). We do not know of any published critical soil P and K concentrations for *Leucadendron* that may assist with interpretation of these soil test data. In relation to K, however, N. A. Maier (unpublished data) found that after 2 years, the application of 25 g K/plant.year did not significantly ($P>0.05$) increase the yield of stems harvested from Silvan Red plants growing in siliceous sand (extractable K values 50–55 mg/kg), compared with plants receiving nil K. We therefore suggest that the critical extractable-K concentration in these soils is low (<50 mg/kg) and the sites were not deficient in K. Similarly, studies with *Protea* species suggest that the P and K requirements for optimum growth are low (Claassens 1981; Maier *et al.* 1995).

For a range of horticultural crops in South Australia, Maier and Robinson (1986) classified soil fertility (on the basis of percentage organic C) as follows: low (<1%), large response to applied N; moderate or normal (1–2%), response to applied N uncertain; high (>2%), no response to applied N. Using this system, the soil at site 1 would be classified as highly fertile and site 2 infertile with regard to N. These data suggest that N fertiliser management should be different at the 2 sites. However, at both sites annual N fertiliser rates were in the range 0–2.5 g/plant, low compared with the amount of N removed in marketable stems alone (Table 2). Reliable soil test interpretation standards are not available for growers to determine the N fertiliser requirements of their plantings or to test the hypothesis with regard to the N fertiliser requirements of the 2 sites. Leaf data for Silvan Red show that during November–April, N concentrations in YFEL were consistently lower at site 2 than site 1 (Fig. 1).

Hannam (1985) presented data showing that for DTPA-extractable Cu, Zn, Mn, and Fe, deficient concentrations were Cu <0.2 mg/kg, Zn <0.5 mg/kg, Mn <1.0 mg/kg, and Fe <2.5 mg/kg. Based on these interpretation standards, the concentrations of these micronutrients in the surface (0–20 cm) soils were adequate (Table 1) and should not have limited yield.

Nutrient composition and removal in harvested stems

For Silvan Red, the amounts of nutrients removed decreased in the order Na > Ca > N > Mg > K > S > P > Mn > Fe > B > Zn > Cu (Table 2). For Safari Sunset the order differed in that more N and Ca were removed than

Table 2. Mean (± s.e.) nutrient concentrations and nutrient removal in 8 kg fresh weight of harvested stems of two *Leucadendron* cultivars

	Nutrient concentration			Nutrient removal		
	Site 1		Site 2	Site 1		Site 2
	Safari Sunset	Silvan Red	Silvan Red	Safari Sunset	Silvan Red	Silvan Red
	(% DW)			*(g)*		
N	0.56 ± 0.03	0.34 ± 0.03	0.31 ± 0.04	28.0 ± 1.6	18.6 ± 3.3	14.6 ± 2.1
P	0.06 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	2.8 ± 0.4	1.8 ± 0.2	2.8 ± 0.2
K	0.28 ± 0.03	0.17 ± 0.02	0.14 ± 0.02	13.8 ± 1.9	9.7 ± 2.3	6.5 ± 0.9
Ca	0.52 ± 0.09	0.35 ± 0.01	0.39 ± 0.02	25.8 ± 4.4	19.2 ± 2.2	17.2 ± 1.0
Mg	0.17 ± 0.02	0.20 ± 0.01	0.17 ± 0.01	8.5 ± 0.9	10.7 ± 0.9	7.5 ± 0.4
Na	0.39 ± 0.03	0.44 ± 0.03	0.51 ± 0.04	19.2 ± 1.5	24.1 ± 3.9	23.4 ± 2.5
S	0.09 ± 0.02	0.09 ± 0.00	0.17 ± 0.00	4.7 ± 0.8	4.8 ± 0.7	7.4 ± 0.4
	(mg/kg DW)			*(mg)*		
B	11 ± 1	13 ± 0	14 ± 0	53 ± 3	67 ± 4	59 ± 3
Cu	2 ± 0	2 ± 0	3 ± 1	10 ± 1	8 ± 1	13 ± 3
Zn	19 ± 2	9 ± 1	10 ± 1	96 ± 13	50 ± 5	44 ± 4
Mn	95 ± 13	79 ± 4	65 ± 4	475 ± 65	423 ± 41	290 ± 23
Fe	42 ± 2	60 ± 13	92 ± 16	209 ± 10	309 ± 59	426 ± 90

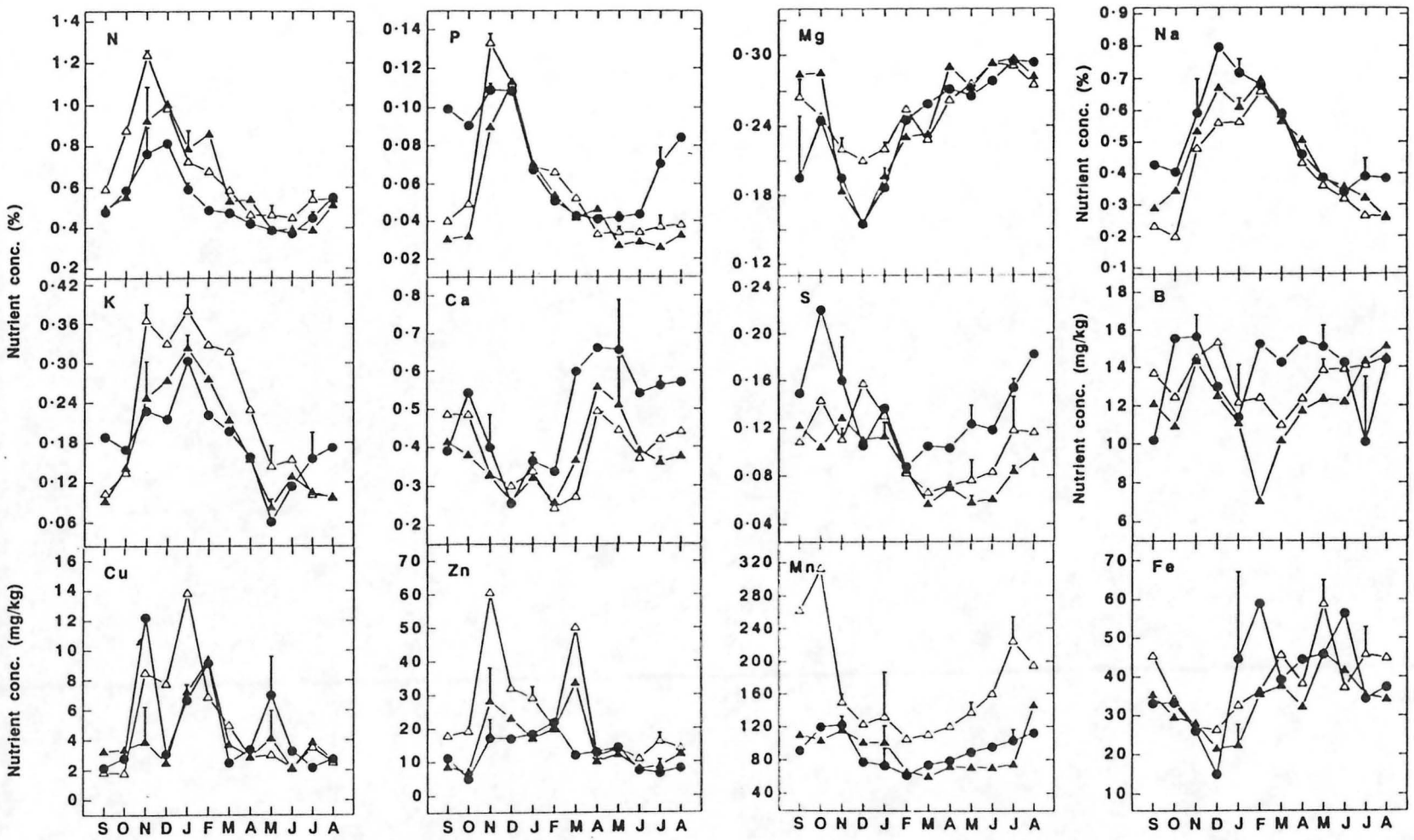

Figure 1. Seasonal changes in nutrient concentrations in youngest fully expanded leaves collected from *Leucadendron* Silvan Red at sites 1 (▲) and 2 (●) and Safari Sunset at site 1 (△). Data presented are mean concentrations over 3 years. Vertical bars indicate representative standard errors.

Na, more K than Mg, and more Zn than B. The amounts of nutrients removed were low compared with other annual or perennial horticultural crops. For example, on the basis of 2600 plants/ha and using the data for Silvan Red at site 1, we estimate the amounts of N, P, K, Ca, and Mg removed in harvested stems to be 48.4, 4.7, 25.2, 49.9, and 27.8 kg/ha, respectively. In contrast, a yield of 50 t/ha of potato tubers removed (kg/ha) 28 P, 242 K, 2 Ca, and 9 Mg (Maier 1986), and 194 t/ha of tomato fruit removed 361 N, 84 P, 615 K, 33 Ca, and 29 Mg (Huett 1985). Because of the relatively high removal rates for Ca and Mg (Table 2), the status of these nutrients should be carefully monitored to avoid deficiencies. This is particularly important when leucadendrons are grown in leached acid sands, which may be low in exchangeable cations. Based on the nutrient removal data, annual applications of Ca and N up to 20–30 g/plant and Mg and K up to 10–15 g/plant should be considered on these soil types.

Larger amounts of Mg, Na, S, Mn, and Zn were removed in harvested stems of the 2 *Leucadendron* cultivars than in stems of Pink Ice. For example, based on the data for Silvan Red and Safari Sunset, the mean ($g \pm$ s.e.) amounts of these nutrients removed were Mg 8.9 ± 0.9, Na 22.2 ± 1.5, S 5.6 ± 0.9, Mn 396 ± 55, and Fe 315 ± 63; for Pink Ice the values were 2.6 ± 0.3, 7.9 ± 2.3, 2.8 ± 0.4, 170 ± 30, and 199 ± 23, respectively. The order of nutrient removal for the 2 *Leucadendron* cultivars also differed from the order reported for Pink Ice (Maier *et al.* 1995).

The concentrations of N, K, Ca, Zn, and Mn in flowering stems of Safari Sunset were higher than those for Silvan Red (Table 2).

Seasonal variation

Seasonal trends in leaf nutrient composition have been reported for many perennial horticultural crops, and factors to explain such trends have been extensively discussed by Cresswell and Wickson (1986), George *et al.* (1989), and Maier *et al.* (1995).

Nitrogen, phosphorus, potassium, sodium. The concentrations of these nutrients were relatively stable during winter, increased rapidly during spring–early summer, and then declined (Fig. 1). The seasonal increase in concentrations corresponded with the spring growth flush (G. E. Barth unpublished data). Jarrell and Beverly (1981) cited the following factors as leading to greater uptake of nutrients and nutrient accumulation: increased root growth and activity; greater transport to tops; greater demand for the element; faster rate of movement to roots through mass flow or diffusion; higher concentration in soil solution. The decrease in concentrations during summer–autumn was consistent with earlier studies (Cresswell and Wickson 1986; George *et al.* 1989), which have reported that mobile nutrients (e.g. N, P, K) usually decline in concentration during the season (spring–autumn). This has been attributed to retranslocation from older or mature leaves to nutrient sinks such as fruit, storage tissue, and newly initiated leaves (Cresswell and Wickson 1986).

Cresswell (1991) suggested the following standards for interpretation of the P status in recently matured leaves of *Leucadendron* cv. Harvest: low–deficient <0.15%, desirable 0.15–0.42%, high 0.42–0.45%, toxic >0.57%. Our data for site 1, the highest yielding site, suggests that depending on sampling time, P concentrations in the range 0.03–0.13% are adequate for high yield and quality of Silvan Red and Safari Sunset. We therefore suggest that the adequate P concentration range in YFEL of field-grown plants is lower than suggested by Cresswell based on greenhouse studies with very young potted plants.

Copper and zinc. Seasonal trends in leaf concentrations of these 2 variably mobile nutrients were similar, with concentrations being very unstable during November–February (Fig. 1) when growth flushing occurred (G. E. Barth unpublished data).

Calcium, magnesium, manganese, iron, boron, sulfur. Studies with other horticultural crops showed that the concentrations of these nutrients, which have low or intermediate mobility in the phloem, tended to increase as the season progressed (Cresswell and Wickson 1986; Stephenson *et al.* 1986). These authors suggested that this trend may reflect leaf age. Our results are consistent with this explanation; for example, since vegetative flushing occurred during November–February, leaves sampled after this were older, and because these nutrients continue to accumulate as leaves age, concentrations increased during the remainder of the season.

The fall in the concentration of these nutrients during spring–early summer (the period of vegetative flushing), may be explained as a growth dilution effect (Jarrell and Beverly 1981).

Cultivar and species differences

For *Leucadendron* cvv. Silvan Red and Safari Sunset, seasonal trends in nutrient composition of YFEL were similar (Fig. 1). Although there were differences in concentrations between cultivars on a given sampling date, they were usually small (except for Mn) and not consistent between all sampling dates. Manganese concentrations were consistently higher in YFEL of Safari Sunset than Silvan Red (Fig. 1). For horticultural crops, published studies on the significance of cultivar differences in relation to the interpretation of plant test data are contradictory. For example, differences in nutrient accumulation between cultivars have been reported for N, P, K, Ca, and Cl^- in grapevine (Robinson and McCarthy 1985); K, Mg, Ca, Fe, and Zn in lemon (Intrigliolo and Starrantino 1988); and N, P, K, Ca, Mg,

Table 3. Mean (± s.e.) and range of nutrient concentrations in youngest fully expanded leaf of *Leucadendron* cvv. Silvan Red and Safari Sunset and *Protea* Pink Ice at site 1

Values are means (± s.e.) and ranges of the monthly concentrations presented in Figure 1 (n = 12)

Values for Pink Ice are based on data presented in Maier *et al.* (1995)

Species	Mean ± s.e.	Range	Mean ± s.e.	Range
	N (%)		*Na (%)*	
Silvan Red	0.61 ± 0.06	0.39–1.00	0.46 ± 0.04	0.26–0.69
Safari Sunset	0.68 ± 0.07	0.45–1.24	0.41 ± 0.05	0.20–0.66
Pink Ice	0.83 ± 0.03	0.71–1.11	0.15 ± 0.01	0.12–0.18
	K (%)		*Zn (mg/kg)*	
Silvan Red	0.18 ± 0.02	0.08–0.32	16 ± 3	7–34
Safari Sunset	0.22 ± 0.03	0.10–0.38	25 ± 5	11–61
Pink Ice	0.30 ± 0.03	0.17–0.45	18 ± 2	12–33
	Ca (%)		*Mn (mg/kg)*	
Silvan Red	0.38 ± 0.03	0.26–0.56	91 ± 8	60–146
Safari Sunset	0.39 ± 0.03	0.24–0.49	169 ± 19	105–313
Pink Ice	0.61 ± 0.04	0.39–0.79	45 ± 2	33–61
	Mg (%)		*Fe (mg/kg)*	
Silvan Red	0.25 ± 0.01	0.16–0.30	33 ± 2	22–46
Safari Sunset	0.25 ± 0.01	0.21–0.29	39 ± 3	26–59
Pink Ice	0.10 ± 0.00	0.08–0.12	46 ± 2	30–55

Fe, and Cu in apple (Tagliavini *et al.* 1992). On the other hand, Cresswell and Wickson (1986) concluded that cultivar differences for N, P, K, Ca, Mg, Zn, Mn, and B in pecan leaves were small and of little practical significance.

In contrast to the relatively small differences in nutrient concentrations between Silvan Red and Safari sunset, concentrations of N, K, Ca, Zn, and Fe in YFEL of Pink Ice were consistently higher, and concentrations of Mg, Na, and Mn consistently lower, than in 2 *Leucadendron* cultivars (Table 3). These data suggest that the interpretation standards developed for *Protea* species may be quite different from those developed for *Leucadendron*, even though the genera are related and are often collectively referred to as Proteas.

Correlations between nutrients

To show the significant (P<0.05) correlations found between nutrients, data for Silvan Red are presented (Table 4). Similar relationships were also found between nutrients in YFEL of Pink Ice (Maier *et al.* 1995). Correlations between nutrients in leaves of plum and cherry were reported by Sanchez-Alonso and Lachica (1987*a*, 1987*b*). The correlations were consistent with the seasonal trends in nutrient concentrations; for example, elements of high mobility (e.g. N, P, K) were positively correlated with each other, and negatively correlated with elements of low mobility (e.g. Ca, Mg, Fe, Mn). Our data (Table 4) are consistent with these conclusions. These correlations may indicate interactions that can affect critical concentrations, induce deficiencies or toxicities,

Table 4. Correlation coefficients (r) for nutrients in the youngest fully expanded leaf of *Leucadendron* Silvan Red

Only relationships significant at P = 0.05 are presented

	N	P	K	Ca	Mg	Na	S
P	0.67						
K	0.74	0.57					
Ca	–0.55		–0.69				
Mg	–0.86	–0.57	–0.67	0.46			
Na	0.63		0.67		–0.78		
S		0.64					
B							0.42
Zn	0.71		0.58		–0.78	0.80	–0.42
Mn						–0.45	
Fe	–0.67		–0.57	0.56	0.55		

and modify growth response, depending on nutrient supply (Lewis *et al.* 1993). For potatoes, Maier *et al.* (1994) and James *et al.* (1994) have discussed the importance of nutrient interactions when using petiole analysis to make nutrient management decisions. They concluded that synergisms and antagonisms between nutrients (e.g. N and P; Cl^- and NO_3^-; K and Mg) need to be considered to ensure correct interpretation.

Sampling for leaf analysis

For perennial crops, the preferred sampling time for diagnostic leaf analysis is when seasonal variation in leaf composition is least and when nutrient levels are best correlated with growth response. However, Cresswell and Wickson (1986) and George *et al.* (1989) reported that selection of a single sampling time suitable for all nutrients is often not possible because of differences in their seasonal trends. Inspection of the seasonal trends presented in Figure 1 shows that concentrations of N, K, Ca, Mg, Na, Cu, Zn, and Fe were fairly stable over June–August. Similarly, concentrations of Ca, Na, and Mn were relatively stable during December–February, when the spring vegetative flush occurred. Samples for P, S, and B analysis may be collected during April–June. However, to establish a plant test for these *Leucadendron* cultivars based on sampling at these times, further experimental work is required to determine the relationships between nutrient concentration and yield response.

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References

Haassens, A. S. (1986). Some aspects of the nutrition of proteas. *Acta Horticulturae* **185**, 171–9.

Cresswell, G. C. (1989). Development of a leaf sampling technique and leaf standards for kiwifruit in New South Wales. *Australian Journal of Experimental Agriculture* **29**, 411–17.

Cresswell, G. C. (1991). Assessing the phosphorus status of proteas using plant analysis. *In* '6th Biennial International Protea Association Conference, Perth, September 1991'. pp. 303–10.

Cresswell, G. C., and Wickson, R. J. (1986). Seasonal variation in the nutrient composition of the foliage of pecan (*Carya illinoensis*). *Australian Journal of Experimental Agriculture* **26**, 393–7.

George, A. P., Nissen, R. J., and Carseldine, M. L. (1989). Effect of season (vegetative flushing) and leaf position on the leaf nutrient composition of *Annona* spp. hybrid cv. Pink's Mammoth in south-eastern Queensland. *Australian Journal of Experimental Agriculture* **29**, 587–95.

Hannam, R. J. (1985). Micronutrient soil test. *In* 'Proceedings of the Soil and Plant Analysis Training Course 1984/85'. (Ed. D. J. Reuter.) Section L. pp. 1–6. (South Australian Department of Agriculture: Adelaide).

Heinsohn, R. D., and Parmenter, N. W. (1986). A preliminary study of the interactions between nitrogen, potassium and phosphorus in the mineral nutrition of seedlings of *Leucadendron salignum* Berg. (Proteaceae). *Acta Horticulturae* **185**, 137–43.

Huett, D. (1985). Plant nutrition. *In* 'Vegetable Growing Handbook 1985'. (Ed. J. Salvestrin.) (Department of Agriculture New South Wales: Sydney.)

Intrigliolo, F., and Starrantino, A. (1988). Nutritional features of 16 clones of lemon. *In* 'Proceedings of the Sixth International Citrus Congress'. (Eds R. Goren and K. Mendel.) March 6–11. Tel Aviv, Israel. (Balaban Publishers: Philadelphia, PA, USA.)

James, D. W., Hurst, R. L., Westermann, D. T., and Tindall, T. A. (1994). Nitrogen and potassium fertilisation of potatoes: Evaluating nutrient element interactions in petioles with response surfaces. *American Potato Journal* **71**, 249–65.

Jarrell, W. M., and Beverly, R. B. (1981). The dilution effect in plant nutrition studies. *Advances in Agronomy* **34**, 197–224.

Lewis, D. C. (1985). Balance Sheet. *In* 'Proceedings of the Soil and Plant Analysis Training Course 1984/85'. (Ed. D. J. Reuter.) Section R. (South Australian Department of Agriculture: Adelaide.)

Lewis, D. C., Grant, I. L., and Maier, N. A. (1993). Factors affecting the interpretation and adoption of plant analysis services. *Australian Journal of Experimental Agriculture* **33**, 1053–66.

Maier, N. A. (1986). Potassium nutrition of irrigated potatoes in South Australia. 2. Effect on chemical composition and the prediction of tuber yield response by plant analysis. *Australian Journal of Experimental Agriculture* **26**, 727–36.

Maier, N. A., Barth, G. E., Cecil, J. S., Chvyl, W. L., and Bartetzko, M. N. (1995). Effect of sampling time and leaf position on leaf nutrient composition of *Protea* 'Pink Ice'. *Australian Journal of Experimental Agriculture* **35**, 275–83.

Maier, N. A., Dahlenburg, A. P., and Williams, C. M. J. (1994). Effect of nitrogen, phosphorus, and potassium on yield and petiolar nutrient concentration of potato (*Solanum tuberosum* L.) cvv. Kennebec and Atlantic. *Australian Journal of Experimental Agriculture* **34**, 825–34.

Maier, N. A., and Robinson, J. B. (1986). Soil analysis for field grown vegetables in SA. South Australian Department of Agriculture Factsheet FS8.83.

Parvin, P. E. (1986). Use of tissue and soil samples to establish nutritional standards in protea. *Acta Horticulturae* **185**, 145–53.

Prance, T. (1992). Fertiliser recommendations—high rainfall pastures: S.A.D.A. balance sheet approach. *In* 'Proceedings of the Soil and Plant Nutrition Training Course Murray Bridge 1992'. (Eds J. Bourne and D. Elliott.) pp. 35–41. (South Australian Department of Agriculture: Adelaide.)

Robinson, J. B., and McCarthy, M. G. (1985). Use of petiole analysis for assessment of vineyard nutrient status in the Barossa district of South Australia. *Australian Journal of Experimental Agriculture* **25**, 231–40.

Sanchez-Alonso, F., and Lachica, M. (1987*a*). Seasonal trends in the elemental content of sweet cherry leaves. *Communications in Soil Science and Plant Analysis* **18**, 17–29.

Sanchez-Alonso, F., and Lachica, M. (1987*b*). Seasonal trends in the elemental content of plum leaves. *Communications in Soil Science and Plant Analysis* **18**, 31–43.

Sparrow, L. A. (1993). A review of fertiliser advice in Australia. *Australian Journal of Experimental Agriculture* **33**, 1067–77.

Stephenson, R. A., Cull, B. W., Mayer, D. G., Price, G., and Stock, J. (1986). Seasonal patterns of macadamia leaf nutrient levels in south east Queensland. *Scientia Horticulturae* **30**, 63–71.

Tagliavini, M., Scudellari, D., Marangoni, B., Bastianel, A., Franzin, F., and Zamborlini, M. (1992) Leaf mineral composition of apple tree: sampling date and effects of cultivar and rootstock. *Journal of Plant Nutrition* **15**, 605–19.

Walkley, A., and Black, T. A. (1934). An examination of the Degtjariff method of determining soil organic matter and a proposed modification of the chromic acid filtration method. *Soil Science* **37**, 29–38.

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Phosphate absorption by excised ordinary and proteoid roots of *Protea compacta* R. Br.

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Over the temperature range of 15 to 35°C the highest phosphate absorption by proteoid roots occurred at 35°C, whereas in the case of ordinary roots absorption increased over the entire temperature range. Desorption revealed that a greater fraction of the absorbed phosphate in ordinary roots apparently occurs in the free space. Phosphate absorption, especially by proteoid roots, was increased by the addition of sugars. Proteoid roots were not consistently characterized by higher phosphate absorption than the ordinary roots. Sugars had no significant effect on oxygen uptake by both types of roots, but proteoid roots displayed a higher oxygen uptake than ordinary roots. The results indicate that proteoid roots are metabolically more active than ordinary roots, and that metabolic processes are to a greater extent involved in ion absorption by proteoid than by ordinary roots.
S. Afr. J. Bot. 1986, 52: 549 – 551

Oor die temperatuurgebied van 15 tot 35°C het die grootste opname van fosfaat deur proteoïede wortels by 25°C plaasgevind, terwyl in die geval van gewone wortels opname oor die hele temperatuurgebied toegeneem het. Desorpsie het aan die lig gebring dat 'n groter fraksie van die opgeneemde fosfaat in gewone wortels klaarblyklik in die vrye ruimte voorkom. Fosfaatopname, veral deur proteoïede wortels, is deur die byvoeging van suikers verhoog. Proteoïede wortels is nie deurgaans deur 'n hoër fosfaatopname as die gewone wortels gekenmerk nie. Suikers het geen betekenisvolle invloed op suurstofopname deur albei wortelsoorte gehad nie, maar proteoïede wortels het 'n hoër suurstofopname as gewone wortels vertoon. Die resultate toon dat proteoïede wortels metabolies meer aktief as gewone wortels is, en dat metaboliese prosesse tot 'n groter mate in ioonopname deur proteoïede as gewone wortels betrokke is.
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Introduction

It is generally assumed that proteoid roots develop especially in infertile soils in order to enhance the absorption of nutrients. Lamont (1976) even regards proteoid roots as a symptom of growth in poor soils.

On account of the effect of the respiratory uncoupler, 2,4-dinitrophenol (DNP) on phosphate and potassium absorption by excised ordinary and proteoid roots of the Proteaceae, as well as on the basis of kinetic analysis of the uptake of the above-mentioned two elements over a wide concentration range, Vorster & Jooste (1986a) concluded that ion uptake by proteoid roots is controlled to a greater extent by metabolic processes than in the case of ordinary roots.

The temperature coefficient (Q_{10}) for active ion absorption is usually two or more (Epstein 1972). It is also generally accepted that metabolic ion absorption is stimulated by carbohydrates and that a relationship between ion accumulation and sugar content of the tissue exists (Pitman, Mowat & Nair 1971).

In an attempt to shed more light on the metabolic nature of ion absorption by proteoid roots, the effects of temperature and carbohydrates on phosphate uptake by excised ordinary and proteoid roots was investigated, as well as oxygen uptake by the two types of roots in the presence and absence of sugars.

Materials and Methods

Root samples of *Protea compacta* R. Br. plants (12 to 18 months old) in dual-compartment gauze bags (to ensure subjection of the two types of roots to identical experimental conditions), were prepared and placed in an intermediate solution (0,5 mmol dm^{-3} $CaSO_4$ at 25°C for 30 min) as previously described (Vorster & Jooste 1986a).

The experimental solutions (minimum volume 250 cm^3 per sample) contained KH_2PO_4 at 0,5 mmol dm^{-3} in a 0,5 mmol dm^{-3} $CaSO_4$ solution. ^{32}P (approximately 83,24 kBq 500 cm^{-3}) was used as tracer. Where the effect of temperature on phosphate absorption was studied, the temperature of the intermediate as well as the experimental solutions was 15,25 and 35°C. In the experiment on the effect of sugars on phosphate absorption, the experimental solutions contained sucrose or fructose at 3 mmol dm^{-3}. Uptake was at 25°C for periods of 15 and 60 min.

Following removal from the experimental solutions, the samples were rinsed for a total of 1 min in a series of four beakers, each containing 200 cm^3 deionized water. Where a desorption treatment was employed (as shown in the relevant tables), the samples were subsequently placed for 30 min in

an aerated desorption medium which was identical to the experimental solution, except that the tracer was omitted and the temperature was kept at 2°C. The pH of the intermediate solution, experimental solutions and desorption media varied between 5,5 and 6,0.

Following these treatments, the samples were allowed to dry on absorbent paper, whereupon the roots were removed from the bags and dried at 80°C for 16 h.

The samples were dry ashed, analysed radiometrically and the phosphate uptake calculated as previously described (Vorster & Jooste 1986a).

Oxygen uptake of ordinary and proteoid roots (1 g samples) was determined with a Gilson respirometer. The following solutions were placed in the main compartment of the reaction flasks: 0,5 mmol dm^{-3} $CaSO_4$ (2,5 cm^3), 0,4 mmol dm^{-3} $NaHCO_3$ (0,5 cm^3) and 100 mmol dm^{-3} sugar solution (2,5 cm^3). In the control treatment the latter was replaced by an equal volume of the $CaSO_4$ solution.

Three to four replicates of each treatment were employed; each experiment was repeated at least twice (on consecutive days). The mean and standard error for each treatment were calculated. Differences between means of more than twice the standard error were regarded as significant.

Results and Discussion

In the case of proteoid roots (Table 1) an increase in temperature from 15 to 25°C generally resulted in higher phosphate absorption, while a decrease occurred with a further rise in temperature to 35°C. Phosphate absorption by the ordinary roots, however, increased over the whole temperature range. This can be regarded as a further indication that metabolic processes are to a greater extent involved in ion absorption by proteoid than by ordinary roots.

The ratio between phosphate absorption by proteoid and ordinary roots (Table 1) illustrates more clearly that in general, relatively more phosphate was absorbed by the proteoid than by the ordinary roots. In addition, desorption was responsible for a greater difference between ordinary and proteoid roots concerning the remaining amount of phosphate — to such an extent that the amount of absorbed phosphate in the ordinary roots, where a desorption treatment had not been applied, was even higher than that in the proteoid roots after an absorption period of 15 min at 25 and 35°C. This indicates that a greater fraction of the absorbed phosphate in ordinary roots apparently occurs in the free space (especially following a shorter absorption period) and is thus capable of being released again — a further indication of greater metabolic absorption by the proteoid roots.

The fact that the amount of phosphate in proteoid roots, following a 15-min absorption period at 35°C, regardless of a desorption treatment, was lower than that in ordinary roots, is possibly related to the already-mentioned suppressing effect of this temperature on absorption by proteoid roots.

Table 2 shows that phosphate absorption by proteoid roots was increased by the addition of sugars. This is illustrated more clearly by the ratio between phosphate absorption in the presence and absence of sugar. The largest stimulation of phosphate uptake in the presence of both fructose and sucrose occurred at the longer absorption period of 60 min. This, together with the fact that sugars had little or no stimulatory effect in the case of ordinary roots, is probably a further indication of the greater involvement of metabolism in ion absorption by proteoid than by ordinary roots.

It should be noted that in this case the amount of absorbed phosphate in proteoid roots was lower or only slightly higher than in ordinary roots (Table 2). It is possible that this could have been due to differences in the initial phosphate status of the two types of roots (Vorster & Jooste 1986a, 1986b). Proteoid roots are short-lived structures, and a seasonal effect might therefore also have been involved, since

Table 1 Phosphate absorption by excised ordinary and proteoid roots at different temperatures. ± Indicates the standard error. The ratio $\frac{\text{P absorption by proteoid roots}}{\text{P absorption by ordinary roots}}$ is also shown

	P Absorption (µg g^{-1} dry mass)											
	15°C				25°C				35°C			
	Proteoid roots		Ordinary roots		Proteoid roots		Ordinary roots		Proteoid roots		Ordinary roots	
Absorption period (min)	With desorption	No desorption	With desorption	No desorption	With desorption	No desorption	With desorption	No desorption	With desorption	No desorption	With desorption	No desorption
15	118 ± 9	155 ± 19	90 ± 19	145 ± 48	244 ± 96	181 ± 76	156 ± 33	219 ± 37	191 ± 68	192 ± 62	268 ± 38	295 ± 42
60	409 ± 118	535 ± 50	210 ± 55	275 ± 24	790 ± 96	600 ± 121	284 ± 78	308 ± 62	580 ± 181	533 ± 101	402 ± 83	437 ± 70

	Ratio $\frac{\text{P absorption by proteoid roots}}{\text{P absorption by ordinary roots}}$					
	15°C		25°C		35°C	
	With desorption	No desorption	With desorption	No desorption	With desorption	No desorption
15	1,31	1,07	1,56	0,83	0,71	0,65
60	1,95	1,95	2,78	1,95	1,44	1,22

Table 2 The effect of sugars on phosphate absorption by excised ordinary and proteoid roots (with desorption). ± Indicates the standard error. The ratio $\frac{\text{P absorption with sugar}}{\text{P absorption without sugar}}$ is also shown

Absorption period (min)	P absorption (μg g^{-1} dry mass)					
	Phosphate		Phosphate + fructose		Phosphate + sucrose	
	Proteoid roots	Ordinary roots	Proteoid roots	Ordinary roots	Proteoid roots	Ordinary roots
15	66 ±29	203 ±55	75 ±34	216 ±58	72 ±26	168 ±29
60	136 ±22	268 ±59	337 ±122	309 ±78	293 ±66	218 ±102
	Ratio $\frac{\text{P absorption with sugar}}{\text{P absorption without sugar}}$					
15			1,14	1,06	1,09	0,83
60			2,48	1,15	2,15	0,81

Table 3 The effect of sugars on oxygen uptake by excised ordinary and proteoid roots. ± Indicates the standard error. The ratio $\frac{\text{oxygen uptake by proteoid roots}}{\text{oxygen uptake by ordinary roots}}$ is also shown

Uptake period (min)	Oxygen uptake (mm^3)					
	Proteoid roots			Ordinary roots		
	Control	Sucrose	Fructose	Control	Sucrose	Fructose
10	19,3± 5,5	13,0± 2,9	21,2± 5,2	12,2±10,3	6,1±0,1	2,0±0,1
20	41,0± 7,0	27,0± 9,7	44,1± 3,5	22,6±12,1	16,2±6,3	10,7±1,6
30	62,0± 7,1	41,2±17,3	65,3± 9,7	33,6±17,7	22,0±7,3	19,6±2,8
40	75,3±10,6	62,1±23,7	85,1±17,1	45,1±26,2	27,5±7,8	27,2±3,3
	Ratio $\frac{\text{oxygen uptake by proteoid roots}}{\text{oxygen uptake by ordinary roots}}$					
10	1,58		2,13		10,60	
20	1,81		1,67		4,12	
30	1,85		1,87		3,33	
40	1,67		2,26		3,13	

this investigation was conducted during the early spring (and was repeated during mid-spring, with the same results), whereas the previously-mentioned experiment (Table 1) was conducted during early winter.

Sugars had no significant effect on oxygen uptake by proteoid and ordinary roots (Table 3). Regardless of the treatment, proteoid roots displayed a higher oxygen uptake than the ordinary roots (Table 3), and this is illustrated more clearly by the ratio between oxygen uptake by proteoid and ordinary roots. This difference in oxygen uptake between the two types of roots was greatest in the presence of fructose.

The higher oxygen uptake by proteoid roots appears to confirm that they are metabolically more active than the ordinary roots and therefore mainly responsible for active ion absorption. Possible seasonal effects can of course not be excluded.

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References

EPSTEIN, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Inc., New York.

LAMONT, B. 1976. Proteoid roots. Root systems in the family Proteaceae and their relevance to horticulture. *Aust. Pl.* 9: 161–164.

PITMAN, M.G., MOWAT, J. & NAIR, H. 1971. Interaction of processes for accumulation of salt and sugar in barley plants. *Aust. J. biol. Sci.* 24: 619–631.

VORSTER, P.W. & JOOSTE, J.H. 1986a. Potassium and phosphate absorption by excised ordinary and proteoid roots of the Proteaceae. *S. Afr. J. Bot.* 52: 277–281.

VORSTER, P.W. & JOOSTE, J.H. 1986b. Translocation of potassium and phosphate from ordinary and proteoid roots to shoots in the Proteaceae. *S. Afr. J. Bot.* 52: 282–285.

ing. Longer chilling periods might cause more buds to sprout on lignified explants. Apparently a short period of low temperature treatment can break the dormancy of buds which are correlatively inhibited. Champagnat (1983) found that buds on single node explants prepared from leafy shoots of *Salix babylonica* L. can be induced to sprout *in vitro* by subjecting the culture to 5°C for two weeks before forcing them at 23°C. Buds on softwood explants of 'Red Sunset' are in a shallower dormancy state than older buds which did not respond to the low-temperature treatment.

Immersion of the feeder leaf of leafy explants into the agar-solidified medium caused significantly more explants to grow than normal explants; however the number of buds per explant that sprouted per growing explant did not differ significantly (Table III). The reason for this is unknown, but the large difference in contact area with the medium between leafy explants (234 ± 8 mm^2) and normal explants (20 ± 2 mm^2) may well be relevant. The highest percentage bud break occurred when the second or third leaf from the top of the explant was used as feeder leaf (Figure 1). Buds distal to the node bearing the feeder leaf sprouted in greater numbers than those subtended by the feeder leaf or buds proximal to the feeder leaf (Figure 2). On intact shoots of apple and rose, bud sprouting is inhibited by the subtending leaf (Theron, Jacobs and Strydom, 1987; Zieslin, Haaze and Halevy, 1976). This is possibly the reason for the poor sprouting of the bud subtended by the feeder leaf. The shoot piece and buds distal to a given bud also contribute to the inhibition of that bud (Zieslin and Halevy, 1976). This may be why fewer buds sprouted when the basal or sub-basal leaf was retained as a feeder leaf. When explants are prepared in this manner the distal buds are farthest from the metabolites absorbed by the feeder leaf, while buds immediately above the bud subtended by the feeder leaf are apparently inhibited by the buds and shoot piece distal to them. The observation that more buds distal to the bud subtended by the feeder leaf sprout, demonstrated the acrotonic behaviour of the shoot piece in terms of bud break. We conclude that the best explants of 'Red Sunset' should be 3-4 nodes long and the basal leaf should be retained as a feeder leaf (Figure 3).

REFERENCES

BEN-JAACOV, J. and DAX, E. (1981). *In vitro* propagation of *Grevillea rosmarinifolia*. *HortScience*, **16**, 309–10.

BEN-JAACOV, J. and JACOBS, G. (1986). Establishing *Protea*, *Leucospermum* and *Serruria in vitro*. *Acta Horticulturae*, **185**, 39–52.

CHAMPAGNAT, P. (1983). Bud dormancy, correlation between organs, and morphogenesis in woody plants. *Fiziologiya Rastenii*, **30**, 587–601.

GORST, J. R., BOURNE, R. A., HARDAKER, S. E., RICHARDS, A. E., DIRCKS, S. and DE FOSSARD, R. A. (1978). Tissue culture propagation of two *Grevillea* hybrids. *Proceedings of the International Plant Propagators' Society*, **28**, 435–46.

MURASHIGE, T. and SKOOG, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, **15**, 473–97.

SEELYE, J. F. (1984). Propagation of NSW Waratah *(Telopea speciosissima)* by tissue culture. *Proceedings of the Internatinal Plant Propagators' Society*, **34**, 403–7.

THERON, K. I., JACOBS, G. and STRYDOM, D. K. (1987). Correlative inhibition of axillary buds in apple nursery trees in relation to node position, defoliation, and promalin application. *Journal of the American Society for Horticultural Science*, **112**, 732–4.

...N, N., HAAZE, H. and HALEVY, A. H. (1976). Components of axillary bud inhibition in rose ...plants. II. The effect of bud position on degree of inhibition. *Botanical Gazette*, **137**, 297–300.

..., N. and HALEVY, A. H. (1976). Components of axillary bud inhibition in rose plants. I. The ...ffect of different plant parts (correlative inhibition). *Botanical Gazette*, **137**, 291–6.

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Factors affecting rooting and auxin absorption in stem cuttings of protea

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SUMMARY

The sensitivity of *Protea* cv. Ivy to supraoptimum concentrations of NAA resulting in tissue die-back from the basal cut surface was used as an indirect method of obtaining information on auxin absorption. Most auxin was absorbed within the first second of treatment, through the basal cut surface while lateral absorbtion was approximately 20-times less effective. The absorption of auxin was also influenced by the physical and chemical properties of the auxin carriers. The existence of an auxin gradient was demonstrated, with the highest concentration at the basal treatment area that decreased to lower concentrations distal from the cut surface. Concentration, treatment time and depth as well as auxin carrier affected rooting of *Protea* stem cuttings.

THE success of propagating plants vegetatively, by cuttings, depends on internal, anatomical and physiological factors as well as external, environmental factors (Chadwick, 1955).

To facilitate the economic vegetative propagation of cuttings, the methods used for rooting should be simple, easy to conduct and of such a nature that they can be commercialized.

The potential of stem cuttings to form adventitious roots can be greatly enhanced by applying auxins. Hartman and Kester (1975) indicated that indole acetic acid (IAA) can be considered a general auxin treatment to root cuttings, using several application methods and carriers to apply the auxins to cuttings. Jacobs and Steenkemp (1975) showed that ethanol and diethylether as solvents and carriers of auxins are much more effective than talc preparations in rooting protea cuttings.

Protea cuttings rooted better following treatment with indole butyric acid (IBA) than with IAA (Rossouw, 1967; Jacobs and Steenkamp, 1975).

Rossouw (1967) and Jacobs and Steenkamp (1975) showed that a concentration of 4 $g\,l^{-1}$ IBA resulted in high rooting percentages in *Mimetus* and *Leucospermum*. Rossouw (1967) also indicated that combinations of IBA and naphthalene acetic acid (NAA) at a concentration of 2 $g\,l^{-1}$ resulted in better rooting with *Mimetus hirtus* and *Protea longiflora*.

The depth of cutting immersion in the auxin solution and the time of treatment also influence rooting. Howard and Nahlawi (1970) showed that immersing the basal cut surface into the auxin preparation increased the rooting of hardwood cuttings in comparison with immersing the whole or most of the cutting into the solution. Comparable results were obtained by Jacobs and Steenkamp (1975) with *P. longiflora*. It was also shown by Howard (1985) that IBA applied to the epidermis of Myrobalan B Plum cuttings was largely ineffective, especially in the presence of IBA at the cut surface. This finding was further demonstrated by Howard (1985) with powder (talc) applications of IBA to the base of plum and apple hardwood cuttings. Application of IBA to the cut base of the stem was more effective than to the proximal epidermis when the powder was preceded by an organic solvent as with cuttings dipped directly into the solvent.

The objective of this study was to determine and evaluate different auxin carriers, time of treatment and depth of treatment into an auxin

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rooting solution for increasing the rooting potential of *Protea* stem cuttings.

MATERIALS AND METHODS

Stem cuttings were selected from a mature clonal commercial planting of *Protea* cv. Ivy, which is a selection from either *P. longiflora* or a hybrid between *P. longiflora* and *P. lacticolor*.

Cuttings were taken from terminal sections of the shoot during the summer and were classified as semi-hardwood.

After sampling the stem cuttings were either processed immediately or stored in plastic bags at +–6°C for not longer than four days. Cuttings were approximately 12 cm long from which the basal two thirds were stripped of leaves. A fresh cut was made before auxin treatment. Except when stated, the basal 10 mm was treated for 5 s with NAA.

On completion of treatment, the stem cuttings were placed in a mist-bed with a rooting medium consisting of 50/50 Irish peat and polystyrene, kept at 20±2°C with heating cables. Intermittent misting to prevent dehydration was controlled by an electronic leaf. The length of dead tissue, as measured from the base of the cutting, and rooting percentage, were determined eight and 60 days after treatment, respectively.

A randomized block design with six replicates of ten cuttings per treatment were used in the experimental layout. Collected data was statistically analysed by arc sin transformation of rooting percentage data and factorial statistical analysis was used to determine significance.

Effect of auxin concentration and carrier

Semi-hardwood stem cuttings were treated with 50% ethanol or 100% diethylether alone or in combination with NAA at concentrations of 5, 10, 15 or 20 g l^{-1}

Effect of treatment time and carrier

Stem cuttings were treated with 10 g l^{-1} NAA in 50% ethanol and 100% diethylether as carriers. The basal 1 or 25 mm of the cutting was immersed in treatment solution for 5 s.

RESULTS AND DISCUSSION

The auxin carriers ethanol and diethylether did not result in any dead tissue in stem cuttings of *Protea* cv. Ivy (Table I) as found with NAA, where the extent of dead tissue was influenced by the type of auxin carrier.

Ethanol as NAA carrier resulted in significantly more dead tissue than when using diethylether. A linear increase in dead tissue resulted from an increase in NAA concentration (Table I). A decrease in rooting percentage was observed for both ethanol and diethylether, as carriers of NAA, with an increase in the concentration of NAA (Table I). NAA with diethylether as carrier resulted in significantly better rooting compared to ethanol.

Control cuttings rooted at the basal cut surface, but auxin treatment produced roots next to the dead tissue.

An increase in treatment time linearly increased the length of dead tissue (Table II). Ethanol as NAA carrier significantly increased the length of dead tissue compared with diethylether, and decreased the percentage rooting (Table II). There was a linear decrease in rooting percentage with an increase in treatment time with ethanol but not with diethylether.

Both ethanol and diethylether, in combination with NAA, significantly increased the length of dead tissue when treatment depth of 25 mm is compared with 1 mm (Table III).

Significantly lower rooting percentages occurred with both carriers of NAA if cuttings were immersed up to 25 mm in the treatment solution compared with 1 mm (Table III).

The concentrated solution immersion method is commonly used to treat stem cuttings with auxins. According to Hartman and Kester (1975), the method involves immersing cuttings into a 50% alcoholic solution of auxin (0.5–10 g l^{-1})to a depth of 5 to 10 mm for approximately 5 s.

If root formation on stem cuttings depends on the amount of auxin absorbed, then the concentration of the auxin solution, the treatment time and treatment depth will be variables determining the amount of auxin absorbed.

Table I.
The effect of different auxin carriers and concentrations of NAA (g l^{-1}) ×10^3 on tissue die back and rooting percentages of Protea *cv. Ivy stem cuttings*

Mean length of dead tissue (mm)	Carrier	0	5	10	15	20
	Ethanol	1.1	56.80	67.80	77.20	92.35
	Diethylether	1.1	32.46	47.83	56.56	50.20
				LSD (P<0.05)		10.38
				(P<0.01)		13.86
Percentage rooted cuttings*	Ethanol	47.88	39.96	25.62	28.77	16.23
	Diethylether	52.15	53.35	49.03	40.15	30.04
				LSD (P<0.05)		5.98
				(P<0.01)		7.78

* Arc sin transformation.

Supraoptimum concentrations of NAA kill the tissues at the base of the cutting, indicating an auxin gradient, with the highest concentration at the base of the cutting (Tables I, II and III). This agrees with Strydom and Hartman (1960) that indole acetic-acid was distributed throughout the cutting within 24 h of treatment with the highest concentration in the basal treated portion. It is postulated that the auxin gradient increases with increase in auxin concentration because an increase from 5 to 20 g l^{-1} NAA, with increments of 5 mg l^{-1}, did not result in a corresponding increase in the length of dead tissue at the base of the cutting (Table I).

Table II shows that most auxin is absorbed within the first second of immersion into the auxin solution. A 20–30 fold increase in treatment time had no corresponding effect on the length of dead tissue compared with 1 s. The rate of auxin uptake decreases rapidly with increases in treatment time.

Rooting is also influenced by the site of auxin application. Howard and Nahlawi (1970) showed that treating the basal cut surface of hardwood plum cuttings gave better rooting than did immersing cuttings deeper into treatment solutions. With an increase in the contact area to auxin by immersing cuttings to a depth of 25 mm, no proportional increase in length of dead tissue could be shown compared with the 1 mm depth treatment (Table III). It can, therefore, be deduced that the basal cutting surface absorbs more auxin than the sides on a unit area/time basis with the immersion treatment technique. These findings can further be substantiated by the results of Howard (1985) with plum and apple cuttings that showed that IBA (talc preparation) applied to the cut base of the stem was more effective than to the proximal epidermis when powder was preceded by an organic solvent, as occurs when cuttings are dipped directly into IBA solution.

Howard (1985) further indicated with Myrobalan B plum cuttings that IBA absorption through the epidermis was largely ineffective, especially in the presence of an

Table II.
The effect of treatment time(s) and auxin carriers of NAA on tissue die back and rooting percentage of stem cuttings of Protea *cv. Ivy*

Mean length of dead tissue (mm)	Carrier	1	20	30
	Ethanol	73.11	82.10	98.58
	Diethylether	46.41	60.90	67.40
			LSD (P<0.05)	−7.27
			(P<0.01)	−9.81
Percentage rooted cuttings*	Ethanol	25.57	27.73	16.88
	Diethylether	40.00	33.96	36.11
			LSD (P<0.05)	−5.04
			(P<0.01)	−6.78

* Arc sin transformation.

TABLE III.
The effect of treatment depth (1 and 25 mm) and auxin carriers of NAA on tissue die back and rooting percentage of Protea *cv. Ivy stem cuttings*

Mean length of dead tissue (mm)	Carrier	1 mm	25 mm
	Ethanol	58.31	96.25
	Diethylether	40.88	65.91
	LSD ($P<0.05$)		−9.51
	($P<0.01$)		−12.93
Percentage rooted cuttings*	Ethanol	34.92	23.40
	Diethylether	43.06	31.89
	LSD ($P<0.05$)		−9.95
	($P<0.01$)		−13.34

* Arc sin transformation.

adequate stimulus from IBA at the basal cut surface.

The fact that the cuttings tend to form roots distal to the dead tissue at the base of the cutting further strengthens the theory that an auxin gradient exists. This implies that there is a concentration of NAA, distal to the dead tissue, that promotes root formation.

The finding that the rooting percentage decreased with an increase in concentration of NAA (Table I), time of treatment (Table II) and treatment depth (Table III) cannot be attributed solely to the supra-optimum concentrations in treated cuttings. An increase in any of the above factors produced a linear increase in the length of dead tissue occurred. This resulted in more dead cuttings and cuttings where dead tissue extended above the rooting medium, thus decreasing the number of cuttings with potential to root. This decrease in cuttings with potential to root was also shown by Jacobs and Steenkamp (1975). They indicated a loss of 84% of cuttings of *Protea* cv. Ivy treated with 8 g l^{-1} NAA compared with only 15% loss with cuttings treated with 1 g l^{-1} NAA. The percentage dead cuttings also increased with an increase in treatment time and depth.

The auxin carriers diethylether and ethanol had no significant effect on rooting or dieback of *Protea* cv. Ivy cuttings. It can be concluded that neither carrier is phytotoxic to protea tissue. The fact that ethanol resulted in more dead tissue on the cutting compared with diethylether for all treatments could be attributed to the ethanol being absorbed faster than diethylether by the cutting. The dieback from treatments using ethanol as carrier is possibly a direct concentration effect. If both carriers are absorbed at the same rate, the less dieback resulting from diethylether can be attributed to the physical characteristics of the chemical. It is, therefore, possible that the higher volatility and immiscibility of diethylether with water that makes the NAA more concentrated at the point of treatment, resulting in a steeper auxin gradient with less tissue dieback (Tables I, II and III). It can be concluded that the higher rooting percentage with diethylether as auxin carrier be attributed to proportionally more cuttings being available to root because of less tissue and/or cutting mortality.

REFERENCES

CHADWICK, L. C. (1985). The choice of shoot: a consideration of the influence of source factors on regeneration. *Report of the 14th International Horticultural Congress*, 215–22.

HARTMANN, H. T. and KESTER, D. E. (1975). *Plant propagation, principles and practices*. 3rd Edition, Prentice-Hall, Engelwood Cliffs, N. J., USA.

HOWARD, B. H. (1985). Factors affecting the response of leafless winter cuttings of apple and plum to IBA applied in powder formulation. *Journal of Horticultural Science*, **60**, 161–8.

HOWARD, B. H. (1985). The contribution to rooting in leafless winter plum cuttings of IBA applied to the epidermis. *Journal of Horticultural Science*, **60**, 153–9.

HOWARD, B. H. and NAHLAWI, N. (1970). Dipping depth as a factor in the treatment of hardwood cuttings with indolybutyric acid. *Annual Report of the East Malling Research Station for* 1970, 201–4.

JACOBS, G. and STEENKAMP, J. C. (1975). Studies on the rooting of Proteaceae stem cuttings. *Annual Report of the Department of Horticultural Science, University of Stellenbosch*, 48–9.

NAHLAWI, N. (1970). The effect of dipping depth and duration of auxin treatment on the rooting of cuttings. *Proceedings of the International Plant Propagating Society*, **20**, 292–300.

ROSSOUW, G. G. (1967). *Propagation of Proteacea from cuttings*. Technical Communication, Department Agricultural Technical Services, No.80. Republic of South Africa.

STRYDOM, D. K. and HARTMANN, H. T. (1960). Absorption, distribution and destruction of IAA in plum stem cuttings. *Plant Physiology*, **35**, 435–42.

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References

Bachelard, E. F., and Sands, R. (1968). Effect of weedicides on starch content and coppicing of cut stumps of manna gum. *Australian Forestry* **32**, 49–54.

Brimblecombe, A. R. (1945). Investigations on starch in living trees of Queensland timber species in relation to control of the powder post beetle. *Australian Timber Journal* **2**, 365–88.

Dubois, M., Gillies, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–6.

Edgar, J. G., Kile, G. A., and Almond, C. A. (1976). Tree decline and mortality in selectively logged eucalypt forests in central Victoria. *Australian Forestry* **39**, 288–303.

Humphreys, F. R., and Kelly, J. (1961). A method for the determination of starch in wood. *Analytica Chimica Acta* **24**, 66–70.

Kile, G. A. (1981*a*). Annual variations in soluble sugars, starch and total food resources in *Eucalyptus obliqua* roots. *Forest Science* **27**, 449–54.

Kile, G. A. (1981*b*). *Armillaria luteobubalina:* a primary cause of decline and death of trees in mixed species eucalypt forests in central Victoria. *Australian Forest Research* **11**, 63–77.

Kile, G. A. (1983). Identification of genotypes and the clonal development of *Armillaria luteobubalina* Watling and Kile in eucalypt forests. *Australian Journal of Botany* **31**, 657–71.

Kile, G. A., and Wade, G. C. (1975). *Trametes versicolor* on apple. II. Host reaction to wounding and fungal infection and its influence on susceptibility to *T. versicolor*. *Phytopathologische Zeitschrift* **82**, 1–24.

Kile, G. A., Kellas, J. D., and Jarrett, R. G. (1982). Factors influencing electrical resistance in stems of *Eucalyptus obliqua, E. globulus* subsp. *bicostata* and *E. viminalis*. *Australian Forest Research* **12**, 139–49.

Leach, R. (1937). Observations on the parasitism and control of *Armillaria mellea*. *Proceedings of the Royal Society*, Series B No. 825 **121**, 561–73.

Leach, R. (1939). Biological control and ecology of *Armillaria mellea* in Britain: biological control. *Transactions of the British Mycological Society* **23**, 320–9.

Priestley, C. A. (1965). A new method for the estimation of the resources of apple trees. *Journal of the Science of Food and Agriculture* **16**, 717–21.

Redfern, D. B. (1968). The ecology of *Armillaria mellea* in Britain: biological control. *Annals of Botany* **32**, 293–300.

Rook, D. A., Swanson, R. H., and Cranswick, A. M. (1977). Reaction of radiata pine to drought. Proceedings of the symposium on Plant and Soil Water Relations, New Zealand Department of Science and Industrial Research Information Series, No. 126, 55–68.

Salisbury, F. B., and Ross, C. W. (1978). 'Plant Physiology'. 2nd edn. (Wadsworth Publishing: Belmont, California.)

Sinclair, R. (1980). Water potential and stomatal conductance of three *Eucalyptus* species in the Mount Lofty Ranges, South Australia: responses to summer drought. *Australian Journal of Botany* **28**, 499–510.

Squire, R. O. (1983). Effects of water and nitrogen on transpiration and growth of *Pinus radiata* D. Don. Ph.D. Thesis, University of Melbourne, Melbourne.

Swift, M. J. (1970). *Armillaria mellea* (Vahl ex Fries) Kummer in central Africa: studies on substrate colonization relating to the mechanism of biological control by ring-barking. In 'Root Diseases and Soil-borne Pathogens'. (Eds T. A. Tousson, R. V. Bega and P. V. Nelson.) pp. 150–2. (University of California Press: Berkeley.)

Wargo, P. M. (1983). How stress predisposes trees to attack by *Armillaria mellea* — a hypothesis. In 'Proceedings of the Sixth International Conference on Root and Butt Rots of Forest Trees, August 25–31, 1983'. (Ed. G. A. Kile.) IUFRO Working Party S2.06.01, pp. 115–9. (CSIRO: Melbourne.)

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Phosphorus Nutrition of Seedlings of the Waratah, *Telopea speciosissima* (Sm.) R.Br. (Proteaceae)

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Abstract

Seedling waratahs, *Telopea speciosissima* (Sm.) R.Br., were grown in acid-washed, phosphate-deficient sand in a glasshouse for 14 weeks under ten phosphate regimes from 0 to 31 mg P kg soil.

Root length increased at the lower levels of applied phosphate, but the dry weights of roots did not, indicating thinner roots at low phosphate levels. Adding phosphate above a level of 0·6 mg P kg soil increased dry matter in shoots and leaf area. Phosphate concentrations in the youngest fully expanded leaf (YFEL) ranged from 0·06% at deficient levels of applied phosphate to 0·4% at the optimal growth level of 3·1 mg P kg soil. Toxicity symptoms were present at phosphate concentrations in shoots of 4·7%.

Cluster roots were found with levels of phosphate addition from 0·7–8 mg P kg soil, and their numbers increased as phosphate was increased from deficient to low levels, and decreased where applied phosphate was adequate for plant growth.

Introduction

Australian soils are particularly low in available phosphorus (Rossiter 1951; Grundon 1972; Donald and Prescott 1975), and the requirement for soil phosphate of many Australian species is considered very low (Specht and Groves 1966; Grundon 1972; Mullette *et al.* 1974). The Proteaceae are perennial, evergreen, slow-growing, usually sclerophyllous plants and are found naturally on nutrient-poor, often highly-leached sands both in Australia and South Africa (Vogts 1958), where they are highly efficient at extracting soil phosphorus and using it for growth. Grundon (1972) found no deficiency symptoms in *Banksia aemula* or *B. oblongifolia* at low levels of applied phosphate, and greater dry matter production for phosphate applied than for white clover or tomato. At high levels of phosphate, banksias developed toxicity symptoms and were considered unable to avoid excessive 'luxury consumption' of phosphorus (Grundon 1972).

Phosphate at the levels of agricultural application have long been regarded as toxic to Proteaceae, and phosphate fertilisers have been used with such caution (Grundon 1972; Thomas 1974; Groves and Keraitis 1976) that it is more commonly believed that Proteaceae dislike applied phosphate. However, this is not always so: for example, Grundon (1972) obtained responses to mixed levels of nitrogen and phosphorus in the four Proteaceae *Banksia robur, B. aeumula, B. oblongifolia* and *Hakea gibbosa*; *Telopea speciosissima* grew better in a high phosphate mix than a low one (Goodwin 1983); and phosphate application increased flower production in *Protea repens* (Lamb and Klaussner 1988). In view of this, I sought to obtain a response curve

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to phosphate for Proteaeae, and chose the waratah, *Telopea speciosissima*. No growth response to phosphate has been established for this species, despite its increasing importance as a commercial flower (Salinger 1985).

Materials and Methods

In this experiment waratah (*Telopea speciosissima*) were grown in a glasshouse in a completely randomised design with 10 replicates of 10 levels of applied phosphate.

Waratah seeds were germinated in the following manner. Seeds were soaked for 10 min in 12% sodium hypochlorite, rinsed twice in sterile distilled water, placed on sterile tissues in a plastic bedding tray, and watered with sterile distilled water. Beds were changed and cleaned regularly in a sterile laminar flow. Germination occurred after 10 days.

After three weeks plants had two or more true leaves and were transplanted into 6 kg free-draining pots which contained a non-sterile, white siliceous acid-washed sand mined at Canning Vale, W.A. This sand overlies Bassendean sand. The sand had been air dried and passed through a 2 mm sieve. It contained <0·001 mg P kg soil. Pots were maintained at field capacity with distilled water.

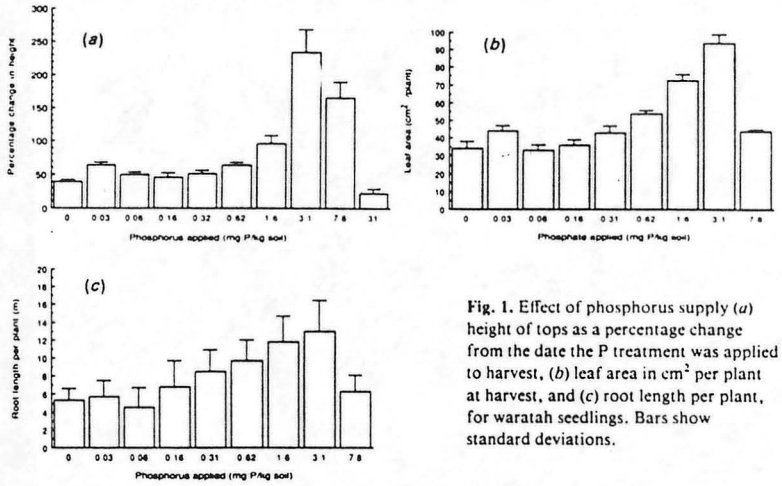

Fig. 1. Effect of phosphorus supply (*a*) height of tops as a percentage change from the date the P treatment was applied to harvest, (*b*) leaf area in cm^2 per plant at harvest, and (*c*) root length per plant, for waratah seedlings. Bars show standard deviations.

After four weeks without added nutrients in pots and 7–8 weeks since germination, the waratah seedlings had chlorotic cotyledons, indicating depletion of their internal nutrient reserves (Stock and Lewis 1984). They were fertilised according to their treatments. Basal nutrients were similar to those of van Staden (1967) and were applied in solution at the following rates (mg kg air-dried soil): $Ca(NO_3)_2.4H_2O$, 118; KNO_3, 51; $MgSO_4.7H_2O$, 49; Fe-EDTA (0·5%), 17; H_3BO_3, 0·57; $MnCl_2.4H_2O$, 0·36; and (as μg/kg air-dried soil): $ZnSO_4.7H_2O$, 22; $CuCl_2.2H_2O$, 11; $Na_2MoO_4.2H_2O$, 4. The treatments were 10 rates of phosphate (0, 0·031, 0·062, 0·155, 0·310, 0·620, 1·55, 3·1, 7·75, 31 mg P/kg air-dry soil, designated hereafter as P_0, $P_{0.03}$, $P_{0.06}$, $P_{0.16}$, $P_{0.31}$, $P_{0.62}$, $P_{1.6}$, $P_{3.1}$, $P_{7.8}$, P_{31}) applied as KH_2PO_4. Pots were completely refertilised a further three times until their harvest at 14 weeks (21–22 weeks after germination).

At harvest, leaf areas were measured (Li-Cor Inc., U.S.A.) and the final stem height (to the apical bud) obtained. Tops were separated into (i) the youngest fully expanded leaf (YFEL), (ii) all other leaves with their petioles, and (iii) stems. Samples were oven-dried at 70° for 2 days and dry weights taken. Sub-samples of the YFEL were digested in nitric-perchloric acid and phosphorus was determined by colorimetric spectrophotometry.

Roots were washed thoroughly in distilled water and stored in acetic alcohol (1:3), after which root lengths (clusters and rest) were measured on a Comair Root Length Scanner (Commonwealth Aircraft Corporation) and the numbers and morphology of cluster roots recorded. Roots were dried at 70°C for 2 days prior to measurement of dry weight.

Analysis of variance was performed on the main data using Genstat V; some data were transformed logarithmally to stabilise the variance of this wild population before an analysis of variance was made. Matrices were used to obtain orthogonal contrasts to compare some select treatments (e.g. P_0 *v.* the rest).

Results

Symptoms

Plants of phosphate levels P_0 to $P_{0.16}$ were stunted, with thick leaves and purpling of leaf tips. Plants grown in $P_{1.6}$ and $P_{3.1}$ appeared most healthy, but those grown in $P_{7.8}$ showed some necrosis. The P_{31} treatment showed severe necrosis of leaf tips and pale, thinner leaves, death of plants occurring by week 5. Due to severe leaf drop the P_{31} level is subsequently absent from much of the data.

Table 1. Effects of phosphate application on dry matter production in tops and roots of waratah seedlings grown for 14 weeks in phosphate-deficient sand

Values are means of 10–20 plants per phosphate treatment

P applied (mg kg soil)	Dry weight (g) YFEL	Total leaves	Stem	Whole root
0	0·076	0·424	0·108	0·213
0·03	0·056	0·485	0·119	0·261
0·06	0·068	0·392	0·105	0·180
0·16	0·070	0·454	0·110	0·207
0·31	0·059	0·510	0·128	0·248
0·62	0·077	0·587	0·153	0·304
1·6	0·083	0·727	0·221	0·397
3·1	0·109	0·915	0·358	0·430
7·8	0·074	0·546	0·192	0·184
l.s.d. (P = 0·05)	0·022	0·128	0·052	0·074

Growth and Dry Matter

Phosphate application to waratahs increased the height of tops (Fig. 1*a*) except at the highest level (P_{31}) which proved very toxic. The greatest increase was at $P_{3.1}$.

Leaf area also increased with phosphate applied from the $P_{0.62}$ to $P_{3.1}$ levels. At $P_{3.1}$ the leaf area was almost triple that of the control. Increasing phosphate beyond $P_{3.1}$ to $P_{7.8}$ halved leaf area ($P < 0·001$) (Fig. 1*b*).

The amount of phosphate received by the seedlings had no effect on the partitioning of dry weight between shoot and root. The dry weights of the whole tops, total leaves and stem showed no real increase until the $P_{0.31}$ or $P_{0.62}$ level (Table 1). All top dry weights were greatest at $P_{3.1}$ and fell on further addition of phosphate. The total dry weight of roots followed the same pattern (Table 1), as did total root length (Fig. 1*c*).

Table 2. Summary of effects of phosphate level on cluster root production in waratah seedlings grown for 14 weeks in phosphate-deficient sand

Values are means of 10–20 replicate plants per phosphate treatment; standard deviations are in parentheses. Average length and average diameter of cluster based on 20 random clusters per phosphate treatment. 1° = primary and 2° = secondary laterals

P applied (mg/kg soil)	Description	Position (laterals)	Number of clusters/plants	% of total root clustered	Mean length of cluster (mm)	Mean diam. of cluster (mm)	Total length of clusters/plant log (cm)	DW clusters/plant log (mg)
0	Dispersed	1° and 2°	12·8 (1·5)	15·8 (1·6)	10 (3)	7 (2)	1·92 (0·24)	0·39 (0·37)
0·03	Dispersed	1° and 2°	15·1 (1·4)	16·9 (1·6)	11 (4)	9 (3)	1·98 (0·21)	0·58 (0·32)
0·06	Dispersed	1° and 2°	9·3 (3·2)	15·8 (2·0)	10 (3)	8 (2)	1·84 (0·50)	0·28 (0·62)
0·16	Dispersed	1° and 2°	17·8 (1·4)	17·4 (1·5)	9 (2)	8 (13)	2·05 (0·25)	0·32 (0·42)
0·31	Dispersed	1° and 2°	31·6 (1·4)	25·7 (1·2)	12 (3)	10 (3)	2·32 (0·14)	0·64 (0·39)
0·62	Dispersed	1° and 2°	35·5 (1·3)	25·1 (1·3)	12 (3)	11 (4)	2·37 (0·18)	0·78 (0·25)
1·6	Dispersed	1° and 2°	26·3 (1·4)	16·2 (1·9)	10 (2)	8 (2)	2·26 (0·28)	0·61 (0·43)
3·1	Dispersed	1° and 2°	5·4 (2·6)	5·4 (5·4)	8 (3)	6 (3)	1·80 (0·41)	−0·09 (0·43)
7·8	Dispersed	1°	3·2 (2·3)	2·0 (2·0)	6 (2)	7 (3)	1·23 (0·22)	−0·60 (0·38)
31	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Cluster roots (Table 2) were well dispersed through the root system. Cluster root numbers were greatest at moderate phosphate applications, severely reduced at $P_{3.1}$ ($P < 0.001$), and totally absent at P_{31}. Cluster root numbers were not related to root length, root dry weight, or shoot dry weight (Table 3) except at low levels of applied phosphate. The average length and diameter of individual clusters did not alter with phosphate level (Table 2), nor did the percentage of the total root length of the whole plant that was clustered. High rates of applied phosphate were detrimental both to the total length of rootlets making up the cluster body and to the dry weight of cluster roots.

Table 3. Correlation coefficients of relationship between cluster root number and (*a*) total root length; (*b*) total root dry weight and (*c*) shoot dry weight, at harvest for seedling waratahs

* Significant at 5% level. ** Significant at 1% level. Degrees of freedom ($n-2$) were variable.

P applied (mg P kg soil)	(*a*) Root length	(*b*) Root dry weight	(*c*) Shoot dry weight
0	0·69*	0·63*	0·67*
0·03	0·56	0·75*	0·41
0·06	0·89**	0·87**	0·84**
0·16	0·66*	0·60*	0·37
0·31	0·53*	0·43	0·65**
0·62	0·55*	0·23	0·24
1·6	0·35	−0·03	0·11
3·1	0·11	0·42	0·43
7·8	0·45	0·07	−0·11

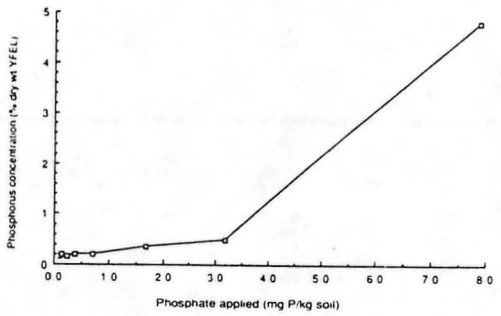

Fig. 2. Effect of phosphorus supply on phosphorus concentration (as % dry matter) in the youngest fully expanded leaf (YFEL) of waratah seedlings grown for 14 weeks in phosphorus-deficient sand. Standard deviations smaller than data points.

Phosphate Concentrations

The phosphate concentration in the YFEL did not respond to increasing levels of applied phosphate until $P_{1.6}$ (Fig. 2). At $P_{3.1}$, which gave the greatest growth, the phosphate concentration was five times the level of P_0. The concentration of phosphate at $P_{7.8}$ — almost 5% — was detrimental to the growth of waratah seedlings.

Discussion

Seedling waratahs showed a growth response to applied phosphate but only after the rate of phosphate exceeded 0·62 mg P/kg soil. Barrow (1977) failed to obtain a response to phosphate in *Banksia grandis* Willd., and he attributed this to its large seed reserve of phosphorus, a low relative growth rate and a very low concentration of phosphorus in the leaves for maximum photosynthesis. In Barrow's experiment, *B. grandis* grew for 22 weeks and showed a 30-fold increase in dry weight but with no variation between phosphorus treatments. In a preliminary experiment (unpublished) I also found no growth response over 22 weeks of treatment to phosphate levels ranging from 0 to 0·55 mg P/kg soil with the West Australian sandplain species *Banksia lemanniana* Meisn. and *B. prionotes* Lindl., even after early death of cotyledons would suggest depletion of nutrient reserves (Stock and Lewis 1984). It was considered that the levels of applied phosphate had been too low for a response in *B. lemanniana* and *B. prionotes*. Indeed, these levels were below the levels where growth responses occurred with waratah.

Others have also found growth responses by waratahs to increased fertility. Parry (1959) reported a good response to occasional dressings of 'blood and bone'. Goodwin (1983) and Nichols and Beardsell (1981) described strong growth responses to increased nutrient supply. Burnett and Mullins (1987) refer to waratahs as 'gross feeders'.

Large variations would be expected in both nutrient requirement and nutrient response within the Proteaceae family. While Australian soils as a whole are nutritionally poor, the deep sands of the Western Australian banksia woodlands are especially low in available phosphorus, and wild populations there would be adapted to much lower and different nutrient levels than plants such as the waratah, which occurs in the Sydney geological basin on clayey sands underlain by sandstone (Crisp and Weston 1987). The basal nutrients of this experiment were similar to those of van Staden (1967) for *Protea magnifica* Link, which is also found on sandstone-derived soils (Rourke 1982).

Biddiscombe *et al.* (1969), working with pasture species, and Brewster *et al.* (1975), working with onions, suggested that some plants can adapt to low phosphate supply by devoting an increased proportion of their total biomass to roots. However my results with wild populations agreed with those of Barrow (1977), who found no change in the root : shoot ratio with phosphorus supply.

The phosphorus concentrations found here in waratah tissue closely agree with those of Grundon (1972), where the chlorosis of toxicity began to be expressed beyond phosphate concentrations of 0·18% (*Banksia robur*), 0·58% (*B. aemula*), 0·24% (*B. oblongifolia*) and 0·94% (*Hakea gibbosa*). Seedling waratahs could tolerate a concentration of 0·4%, as measured in the YFEL. These results for phosphorus concentrations are much higher than those given by Parvin *et al.* (1973) who describe plants with P toxicity symptoms of necrotic red leaves of *Protea neriifolia* R.Br. as having P concentrations as low as 0·05–0·10% in 'new growth' (but possibly not the YFEL) on three Hawaiian soils. Wallace *et al.* (1986) point out that comparing leaf nutrient status between plants on a dry-weight basis might be complicated by the high level of cutinisation, sclerification and silicification in sclerophyllous leaves (Small 1973) which are typical of Proteaceae.

The findings for cluster roots differed from other workers, who have found cluster roots present only at low levels of phosphate (Jeffrey 1967; Grundon 1972; Lamont 1972; Barrow 1977). Barrow (1977) attributed the failure of his banksias to respond to phosphorus to a lack of cluster roots in his experiment. However, in my results considerable numbers of cluster roots were present from 0 to 0·62 mg P/kg despite no growth response at these levels. Again, in a preliminary experiment, *Banksia* spp. produced cluster roots prolifically, with no growth response to applied phosphate. Waratah seedlings produced cluster roots at phosphate concentrations as high as 7·8 mg P/kg. The number of cluster roots actually increased as phosphate application increased from the deficient (P_0) range to the low ($P_{0.6}$) range, and decreased where phosphate became adequate for plant growth. Lamont (1972) showed that a phase can exist in which an increase in the nutrient status of the plant is accompanied by an increase in cluster root production. That cluster roots were present where P toxicity symptoms began to develop and where plant growth was significantly reduced, and that cluster length and diameter did not vary with increasing phosphate applied, needs further investigation.

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References

Barrow, N. J. (1977). Phosphorus uptake and utilization by tree seedlings. *Australian Journal of Botany* **25**, 571–84.

Biddiscombe, E. F., Ozanne, P. J., Barrow, N. J., and Keay, J. (1969). A comparison of growth rates and phosphorus distribution in a range of pasture species. *Australian Journal of Agricultural Research* **20**, 1023–33.

Brewster, J. L., Bhat, K. K. S., and Nye, P. H. (1975). The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. II. The growth and uptake of onions in solutions of constant phosphate concentration. *Plant and Soil* **42**, 171–95.

Burnett, J., and Mullins, M. G. (1987). Cultivation of the waratah as a commercial flowercrop. *In* 'Waratahs — their Biology, Cultivation and Conservation'. (Ed. J. A. Armstrong.) pp. 45–50. Australian National Botanic Gardens Occasional Publication No. 9.

Crisp, M. D., and Weston, P. H. (1987). Waratahs — how many species? *In* 'Waratahs — the Biology, Cultivation and Conservation'. (Ed. J. A. Armstrong.) pp. 3–15. Australian National Botanic Gardens Occasional Publication No. 9.

Donald, C. M., and Prescott, J. A. (1975). Trace elements in Australian crop and pasture production, 1924–1974. *In* 'Trace Elements in Soil-Plant-Animal Systems'. (Eds D. J. D. Nicholas and A. R. Egan.) pp. 7–37. (Academic Press: New York.)

Goodwin, P. B. (1983). Australian natives — fertilizing container-grown plants. *Australian Horticulture* **81**, 57–65.

Groves, R. H., and Keraitis, K. (1976). Survival and growth of seedlings of three sclerophyll species at high levels of phosphorus and nitrogen. *Australian Journal of Botany* **24**, 681–90.

Grundon, N. J. (1972). Mineral nutrition of some Queensland heath plants. *Journal of Ecology* **60**, 171–81.

Jeffrey, D. W. (1967). Phosphate nutrition of Australian heath plants. I. The importance of proteoid roots in *Banksia* (Proteaceae). *Australian Journal of Botany* **15**, 403–11.

Lamb, A. J., and Klaussner, E. (1988). Response of the fynbos shrubs *Protea repens* and *Erica plukenetii* to low levels of nitrogen and phosphorus application. *South African Journal of Botany* **54**, 558–64.

Lamont, B. (1972). The effects of soil nutrients on the production of proteoid roots by *Hakea* species. *Australian Journal of Botany* **20**, 27–40.

Mullette, K. J., Hannon, N. J., and Elliott, A. G. L. (1974). Insoluble phosphorus usage by *Eucalyptus*. *Plant and Soil* **41**, 199–205.

Nichols, D. G., and Beardsell, D. B. (1981). The response of phosphorus-sensitive plants to slow release fertilizers in soil-less potting mixes. *Scientia Horticulturae* **15**, 301–9.

Parry, P. J. (1959). How to grow waratahs. *Australian Plants* **1**, 2.

Parvin, P. E., Criley, R. A., and Bullock, R. M. (1973). Proteas: developmental research for a new cut flower crop. *HortScience* **8**, 299–303.

Rossiter, R. C. (1951). Studies on the nutrition of pasture plants in the south-west of Western Australia. 2. Visual symptoms of mineral deficiencies in the Dwalganup strain of *Trifolium subterraneum* L. *Australian Journal of Agricultural Research* **2**, 14–23.

Rourke, J. P. (1982). 'The Proteas of Southern Africa.' (Centaur Publications: Johannesburg.)

Salinger, J. P. (1985). 'Commercial Flower Growing.' (Butterworths Horticultural Books: Wellington, New Zealand.)
Small, E. (1973). Xeromorphy in plants as a possible basis for migration between arid and nutritionally-deficient environments. *Botaniska Notiser* **126**, 534–9.
Specht, R. L., and Groves, R. H. (1966). A comparison of the phosphate nutrition of Australian heath plants and introduced economic plants. *Australian Journal of Botany* **14**, 201–21.
Stock, W. D., and Lewis, O. A. M. (1984). Uptake and assimilation of nitrate and ammonium by an evergreen fynbos shrub species *Protea repens* L. (Proteaceae). *New Phytologist* **97**, 261–8.
Thomas, M. B. (1974). Research on the nutrition of container-grown Proteaceae plants and other nursery stock. *Proceedings, International Plant Propagation Society* **24**, 313–25.
Van Staden, J. (1967). Deficiencies of major nutrient elements in *Protea cynaroides* Linn., grown in sand culture. I. Foliar symptoms of deficiencies. *Journal of the South African Botanical Society* **33**, 59–64.
Vogts, M. M. (1958). 'Proteas: Know Them and Grow Them'. (Afrikaanse Persboekhandel. (Edms) Beperk.: Johannesburg.)
Wallace, I. M., Dell, B., and Loneragan, J. F. (1986). Zinc nutrition of jarrah (*Eucalyptus marginata* Donn ex Smith) seedlings. *Australian Journal of Botany* **34**, 41–51.

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Species Richness of Overstorey Strata in Australian Plant Communities — the Influence of Overstorey Growth Rates

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Abstract

The species richness (number of tree and tall shrub species per hectare) of overstorey strata is examined in tropical, subtropical and temperate climax plant communities of Australia. Species richness (N) is shown to increase as the evaporative coefficient (k) of the sampling site increases from semiarid climates (k = 0·035–0·045) to perhumid climates (k = 0·075–0·100):

Tropical:	$\log N = 17{\cdot}38k + 0{\cdot}40$.
Subtropical:	$\log N = 25{\cdot}40k - 0{\cdot}60$.
Temperate:	$\log N = 8{\cdot}90k + 0{\cdot}09$.

Species richness of overstorey strata is highest in the tropics (N = 138 when k = 0·100), followed by the subtropics (N = 73 when k = 0·100), with only a small number of overstorey species being associated with temperate communities (N = 8–10 when k = 0·100).

Species richness of the overstorey is positively related to the annual shoot growth (vertical component) of the foliage canopy as it regenerates after disturbance. The tendency to dominance of only a few overstorey species in temperate communities may be enhanced by rapid stem growth (current annual growth increment) of the plant community due to lower cellular metabolism and respiration in stems and roots in cooler climates compared with those in the tropics.

Trees and tall shrubs appear to be excluded as overstorey components when the respiratory coefficient (c) approaches 0·030 and the mean annual temperatures (of snow-free localities) are less than 13°C.

Introduction

In a review of the literature on species diversity in plant communities, Huston (1979) was puzzled by the apparently opposing views concerning the influence of productivity on species richness. A number of ecologists (Connell and Orias 1964; Pianka 1966; MacArthur 1969) had produced evidence that species diversity was positively correlated with productivity. In contrast, other authors (Yount 1956; Margalef 1969) had shown that species diversity decreased as productivity increased.

It is this paradox of opposing views on the influence of productivity on species richness that this and the following paper (Specht and Specht 1989*b*) attempt to address. In the first paper, the paradox is explored by an analysis of the rate of development of the overstorey canopy of forest communities and its influence on the species richness of the overstorey. In the second paper, the effect of shading from the developing overstorey cover in sclerophyll plant communities (heathlands, heathy shrublands/woodlands/open forests) on the species richness of the understorey is examined.

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EFFECTS OF COLD STORAGE METHODS ON VASE LIFE AND PHYSIOLOGY OF CUT WARATAH INFLORESCENCES (*TELOPEA SPECIOSISSIMA*, PROTEACEAE)

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ABSTRACT

Faragher, J.D., 1986. Effects of cold storage methods on vase life and physiology of cut waratah inflorescences (*Telopea speciosissima*, Proteaceae). *Scientia Hortic.*, 29: 163—171.

The effects of long-term, dry cold storage on the vase life and physiology of waratah inflorescences (*Telopea speciosissima* R.Br.) was investigated. Storage at 0 or 2°C and 100% RH for 2 weeks did not reduce the subsequent vase life at 20°C, but storage at 0°C for 4 weeks reduced it from 8 days in fresh inflorescences to less than 6 days. Storage at 4°C for 4 weeks further shortened vase life to 3 days, and storage at 80% RH reduced vase life compared with storage at 100% RH. Harvest maturity had no significant effect on post-storage vase life. Storage also reduced subsequent flower opening on the inflorescence. The shorter vase life of cold-stored waratahs was associated with earlier changes in properties of the inflorescence which normally accompanied senescence of fresh waratahs; decreases in water uptake and flower and bract fresh weights, and increases in nett water loss, perianth abscission and ethylene production by flowers. That is, cold storage advanced subsequent senescence of the inflorescence at 20°C, apparently because ageing had occurred during cold storage.

Keywords: flower senescence; flower storage; protea.

INTRODUCTION

Waratah (*Telopea speciosissima* R.Br., Proteaceae) is a promising new cut-flower crop, particularly in Australia where it is indigenous. Future marketing of waratahs may require several weeks cold storage to lengthen the period of supply and to allow economical sea shipment to export markets. The response of waratahs, and other Proteaceae flowers, to long-term cold storage has not been thoroughly investigated. Waratahs were stored at 0.5°C for 9—10 days and the subsequent vase life was shortened by 30% (Worrall, 1983). Results of experiments with *Protea* sp. are variable and inconsistent. Meynhardt (1976) reported that several species could

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be successfully stored at 2°C for 6 weeks, but Jacobs (1981) reported that some of those listed by Meynhardt (1976) and other species had an unsatisfactory vase life after 3 weeks at 2°C. It has also been suggested that higher storage temperatures, of 2–8°C, may be more appropriate for *Protea* (Halevy and Mayak, 1981).

The aims of the present study were: (1) to investigate the effects of cold storage, and the components of the storage technique (temperature, humidity and duration), on the vase life and quality of the inflorescence; (2) to investigate the possible physiological mechanisms affecting vase life and flower quality after cold storage.

MATERIALS AND METHODS

Inflorescences. — The waratah inflorescence is a terminal raceme of 150–200 red flowers surrounded by red bracts, and is illustrated by Faragher (1986). In this work, the term inflorescence includes flowers, bracts, stems and leaves, and the term flower refers to the individual flowers of the raceme. Inflorescences were cut from up to 40 seed-grown plants and transported to the laboratory, with stems in water, within 1 h. Inflorescences were cut when between 25 and 33% of the flowers were open, as this was the normal stage of development at commercial harvest. In an experiment to test the effect of maturity on response to cold storage, inflorescences were cut when 6, 27 or 48% of flowers had opened. Stems were cut to 30 cm in length, one-third of the leaves were removed and the inflorescences were dipped in Rovral fungicide (iprodione, May and Baker, Australia, 1 g l^{-1}).

Experimental treatments. — Several independent experiments were carried out to investigate the effects of cold storage duration, humidity and temperature and the effect of flower maturity. In each experiment, fresh, unstored flowers were the controls and 6–8 replicate inflorescences were used. The storage conditions for each experiment are shown in the tables. Cold storage was without water. The temperatures used were 0.0 ± 0.5, 2 ± 1 or 4 ± 1°C. Humidities were 85 ± 5% inside a fibreboard carton, or close to 100% with free water present in a loosely closed polyethylene bag (low-density polyethylene ca. 38 μm thick). Storage durations were 0 (fresh), 2 or 4 weeks. After cold storage, stems were re-cut to 3 cm and placed in deionised water. For observation of vase life, flower opening and changes in physiological properties, inflorescences were held in water at 20 ± 1°C and 60% RH, with continuous white fluorescent light of 5 W m^{-2} irradiance. In one experiment the effect of chlorine bactericide on vase life after cold storage was investigated, and sodium dichloroisocyanurate was added to the vase water at 25 mg l^{-1} available chlorine. The vase water, or chlorine solution, was changed every second day.

Measurements. — The end of vase life was defined as when there was readily discernible wilting and color change from bright-red to blue-red in at least one-third of the flowers and all of the bracts. Generally, wilting and blueing occurred at the same time, and these changes occurred in the bracts when they were also visible in one-third of the flowers. Flower opening was expressed as the percentage of flowers on the inflorescence which had opened. Fresh weight and ethylene production were measured on representative bracts and the outermost (oldest) flowers, which were sampled from the inflorescence at intervals during vase life. Water uptake, transpiration and nett water loss (transpiration minus uptake) by the inflorescence were measured. Measurements were made as described previously (Faragher, 1986). Perianth abscission was evaluated in two ways, firstly by noting the time when it was first visible in the oldest flowers, and secondly by calculating an abscission rating, i.e. the percentage of flowers in which abscission had occurred in a sample of two flowers from each of eight inflorescences

RESULTS

Effects of cold storage on vase life, flower opening and quality. — Vase life was not shortened by 2 weeks storage, but was shortened when storage duration was increased to 4 weeks (Table I). Vase life was shortened when

TABLE I

Effect of cold storage duration on vase life of waratahs. Storage was at 2°C and 100% RH. Means followed by a different letter differ significantly ($P < 0.05$, Duncan's multiple range test)

Storage duration (weeks)	Vase life (days)
0	8.3 a
2	8.2 a
4	4.3 b

TABLE II

Effect of humidity in cold storage on vase life of waratahs. Storage was at 2°C for 2 weeks. Means followed by a different letter differ significantly ($P < 0.05$, Duncan's multiple range test)

Storage treatment	Vase life (days)
No storage	8.3 a
100% RH	8.2 a
80% RH	6.5 b

storage humidity was reduced from 100 to 80% RH (Table II) and when storage temperature was increased from 0 to 4°C (Table III). Inflorescence maturity (6, 27 or 48% flowers open when cut) had no significant effect on vase life after cold storage for 4 weeks at 2°C and 100% RH ($P \leq 0.05$, Duncan's multiple range test, data not shown). Cold storage reduced subsequent flower opening, compared with unstored inflorescences, in waratahs cut at three maturities (Table IV). Other minor effects of cold storage on quality were the occasional fungal infection despite fungicide treatment, and wilting of the inflorescence during storage at 80% RH, although the inflorescences regained turgor after transfer to water.

TABLE III

Effect of cold-storage temperature on vase life of waratahs. Storage was for 4 weeks at 100% RH. Means followed by a different letter differ significantly ($P < 0.05$, Duncan's multiple range test)

Storage treatment	Vase life (days)
No storage	7.9 a
0°C	5.8 b
4°C	3.1 c

TABLE IV

Effect of cold storage on flower opening during vase life of waratahs. Inflorescences were cut at three stages of maturity, with 6, 27 or 48% of flowers open. Storage was for 4 weeks at 2°C and 100% RH. For each maturity, mean values followed by a different letter differ significantly ($P < 0.05$, Duncan's multiple range test)

Inflorescence maturity (% flowers open)	Storage treatment	Flower opening (%)
6	No storage	45 a
	Storage	16 b
27	No storage	60 a
	Storage	40 b
48	No storage	72 a
	Storage	53 b

Effects of cold storage on physiology of the inflorescence. — The effects of storage duration on the subsequent physiology of the inflorescence during vase life at 20°C are shown in Fig. 1. After 2 weeks storage, the fresh weights of flowers and bracts during vase life were not significantly less than those of fresh, unstored waratahs (comparison of means by *t*-test, at $P \leq 0.05$, at the same sampling times) (Fig. 1A,B). However, after 4

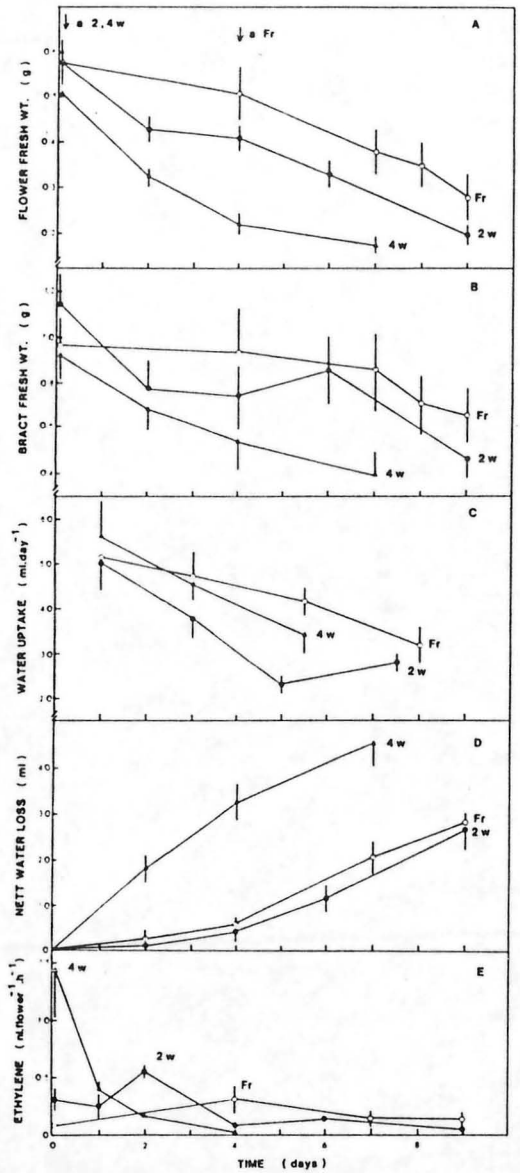
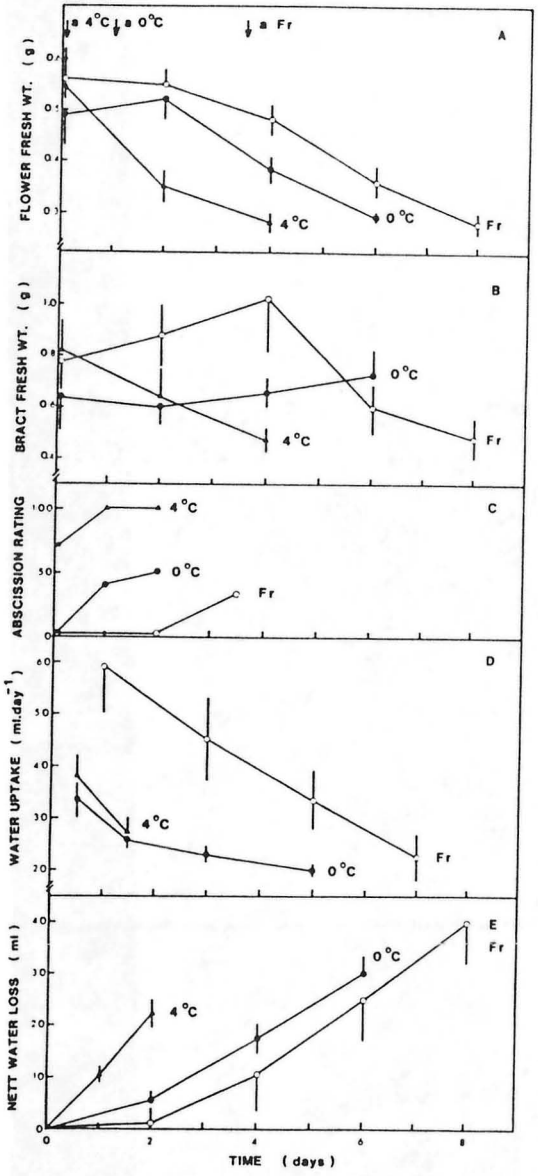


Fig. 1. Effects of storage duration on changes in the waratah inflorescence during subsequent vase life at 20°C. ○, fresh not stored (Fr); ●, 2 weeks storage at 2°C and 100% RH (2 w); ▲, 4 weeks storage (4 w). Values are means of 8 replicates ± standard errors. A. Flower fresh weight, outermost flowers. The time when perianth abscission was first visible is indicated by arrows and the letter a. B. Bract fresh weight. C. Water uptake by the inflorescence. D. Nett water loss (cumulative) from the inflorescence. E. Ethylene production by flowers.



weeks storage, fresh weights were significantly less than those in fresh, unstored waratahs (Fig. 1A,B). Perianth abscission was visible immediately after removal from both 2 and 4 weeks storage, but did not occur in fresh flowers until after 4 days at 20°C (Fig. 1A). Water uptake by the influorescence was less after 2 weeks storage than in fresh inflorescences, but after 4 weeks storage uptake was similar to that in the fresh inflorescences (Fig. 1C).

The cumulative nett water loss from the inflorescences increased with time at 20°C (Fig. 1D). Inflorescences stored for 2 weeks had the same water loss as fresh inflorescences, but those stored for 4 weeks had a much greater nett water loss (Fig. 1D). The increase in ethylene production by flowers during senescence was advanced and stimulated by prior storage (Fig. 1E). After 4 weeks storage, the maximum rate occurred immediately after transfer from 2 to 20°C, and was approximately five times the maximum rate in fresh flowers.

The effects of storage temperature on the physiology of inflorescences at 20°C are shown in Fig. 2. As storage temperature was increased from 0 to 4°C, the fresh weight of flowers and bracts decreased more rapidly during vase life (Fig. 2A,B). This was associated with earlier perianth abscission as storage temperature increased (Fig. 2A). The percentage of abscised perianths in a sample of the oldest flowers from inflorescences (abscission rating) increased most rapidly in stored flowers, particularly in those stored at 4°C (Fig. 2C). Water uptake was less after storage at both 0 and 4°C than in fresh inflorescences (Fig. 2D). Nett water loss was not significantly greater in inflorescences stored at 0°C than in fresh ones, but it was greater after storage at 4°C (Fig. 2E).

Chlorine bactericide added to the vase water increased the vase life of fresh waratahs but did not significantly increase vase life after cold storage for 4 weeks at 2°C and 100% RH ($P < 0.05$, t-test, data not shown).

DISCUSSION

Cold storage was successfully used to hold waratahs without loss of vase life for 2 weeks, and with a small, acceptable loss of life for 4 weeks. The loss of vase life after extended cold storage was the only major, adverse effect of storage. Cold storage also reduced flower opening, but this is probably not a commercial disadvantage. Vase life was very sensitive to

Fig. 2. Effects of storage temperature on changes in the waratah inflorescence during vase life at 20°C. ○, fresh not stored (Fr); ●, storage at 0°C and 100% RH for 4 weeks; ▲, storage at 4°C. Values are means of 8 replicates ± standard errors. A. Flower fresh weight, outermost flowers. The time when perianth abscission was first visible is indicated by arrows and the letter a. B. Bract fresh weight. C. Perianth abscission rating, outermost flowers (see Materials and Methods for calculation). D. Water uptake by the inflorescence. E. Nett water loss (cumulative) from the inflorescence.

storage duration, temperature and humidity. Storage for 4 weeks under the optimum conditions of 0°C and 100% RH shortened vase life from 8 days in fresh waratahs to 6 days. This 2-day, 25%, loss of life is probably the maximum which could be tolerated commercially, and so 4 weeks is probably the maximum feasible storage life. It may be valuable to know the more detailed effects of storage duration, for example the effects of 3, 4 and 5 weeks storage. When storage temperature was higher than 0°C, or humidity reduced from 100 to 80% RH, vase life was markedly shortened, so in practice the careful use of optimum storage conditions will be critical for subsequent life of waratahs. It has been assumed that the major effect of storage in polyethylene bags was to increase the humidity to close to 100% RH, but changes in O_2 or CO_2 concentrations in the bags cannot be ruled out. The loss of vase life in my experiments was less than that reported by Worrall (1983), who found a 30% loss of life after 10 days storage at 0.5°C. The slower loss of life in my experiments could be due to higher storage humidity, use of more mature inflorescences, or other differences between the inflorescences. The actual vase life of 6—8 days appears short for flowers of the Proteaceae, which are assumed to have long lives. This is probably because waratahs do senesce more rapidly than other Proteaceae flowers, and because relatively severe conditions were used to measure vase life with continuous 20°C, air circulation and light.

Senescence of cold-stored waratahs during vase life at 20°C followed the same pattern as senescence in fresh, unstored inflorescences, but the events were advanced. The sequence of senescence events appears to involve decreased water uptake, nett water loss, perianth abscission and increased ethylene production as initial events, followed inevitably by loss of fresh weight in flowers and bracts, blueing and the end of vase life.

The regulation of the initial events of waratah senescence is unclear. Nett water loss is an expected result of decreased water uptake. However, in at least one case, water loss increased but uptake did not decrease (4 weeks storage, Fig. 1C,D), so it appears that transpiration was increased, possibly as a result of increased cell membrane permeability during senescence (Mayak and Halevy, 1980; Faragher and Mayak, 1984). Abscission could be caused by nett water loss, or by the increased ethylene production in flowers (Halevy and Mayak, 1981; Reid, 1985). Ethylene promotes senescence of other flowers (Mayak and Halevy, 1980; Halevy and Mayak, 1981), but its role in waratahs remains to be determined.

There was some evidence that ageing and senescence had proceeded during cold storage since abscission had started, flower ethylene production rates had increased and water uptake had decreased at the time of transfer from cold storage to 20°C. This suggests that the major effect of cold storage on the physiology of the waratah inflorescence was to allow ageing to occur in storage, so that when it was transferred to 20°C, ageing was already advanced and thus vase life was shortened. There was

one other effect of cold storage on waratahs, which was to increase the rate of ethylene production by flowers after transfer to 20°C. This also occurs in roses (Faragher and Mayak, 1984) and several fruits, and it appears to be a typical response of some plant tissues to low temperatures (Wang, 1982). However, the significance of this ethylene in waratah flower senescence is not known.

Chlorine bactericide treatment of vase water increased the life of fresh inflorescences, probably because it increased water uptake (Faragher, 1986). However, it did not increase vase life or water uptake of cold-stored waratahs. Thus, it appears that the effect of cold storage, which reduces water uptake, is somewhat different to the effect of ageing on uptake in freshly cut waratahs.

It is concluded that dry cold storage of waratah inflorescences is feasible for a period of up to 4 weeks at 0°C and 100% RH, with only a 25% loss of vase life. At higher storage temperatures or lower humidities, loss of vase life would be greater unless storage time was shortened. The effects of cold storage are primarily due to advanced senescence, probably as a result of continued ageing during storage.

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REFERENCES

Faragher, J.D., 1986. Post-harvest physiology of waratah inflorescences (*Telopea speciosissima*, Proteaceae). Scientia Hortic., 28: in press.

Faragher, J.D. and Mayak, S., 1984. Physiological responses of cut rose flowers to exposure to low temperature: changes in membrane permeability and ethylene production. J. Exp. Bot., 35: 965—974.

Halevy, A.H. and Mayak, S., 1981. Senescence and post-harvest physiology of cut flowers. Part 2. Hortic. Rev., 3: 59—143.

Jacobs, G., 1981. Post-harvest handling of proteas. In: P. Mathews (Editor), Growing and Marketing of Proteas, Vol. 1. Proteaflora Enterprises, Melbourne, pp. 40—51.

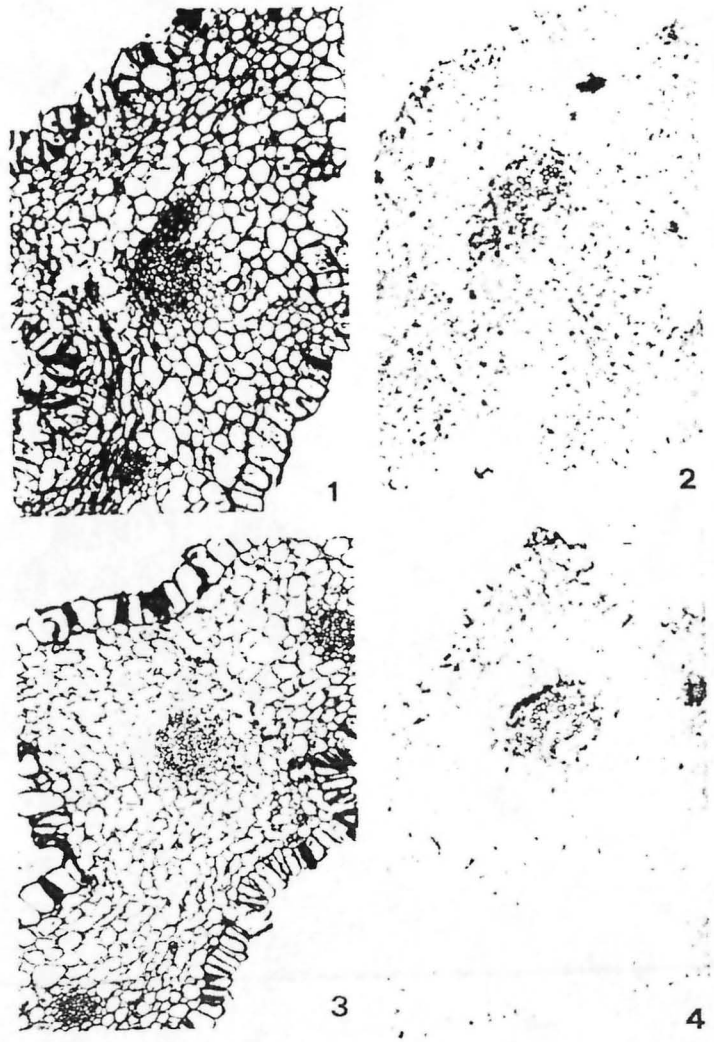
Mayak, S. and Halevy, A.H., 1980. Flower senescence. In: K.V. Thimann (Editor), Senescence in Plants. CRC Press, Boca Raton, FL, pp. 131—156.

Meynhardt, J.T., 1976. Proteas — picking and handling. In: Farming in South Africa, Flower Ornamental Shrubs and Trees Series. No. B5/1976. Dep. Agric. Tech. Serv., Pretoria, S. Africa, 3 pp.

Reid, M.S., 1985. Ethylene and abscission. HortScience, 20: 45—50.

Wang, C.Y., 1982. Physiological and biochemical responses of plants to chilling stress. HortScience, 17: 173—186.

Worrall, R.J., 1983. Growing waratahs commercially. Aust. Hortic., 81: 101—103.



Figs. 1-4 - Cross section of the basal portion of Carnation petals. 1 & 2 'Alice'; 3 & 4 'Astor'. Left unextracted, right after pectins removal. 116x.

LEAF BLACKENING IN CUT PROTEA EXIMIA: IMPORTANCE OF WATER RELATIONS

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Abstract

Leaf blackening occurs at different rates among different species of P r o t e a used as cut flowers. P r o t e a e x i m i a, the Rose-spoon protea, shows very rapid leaf blackening. The use of Floralife preservative before and after simulated shipping, shipping temperatures, and packaging in plastic bags was investigated.

The use of Floralife (20 g/l) delayed leaf blackening and wilting of the flower over the use of deionized water only for all temperatures. The use of a preservative allowed warmer shipping temperatures, as at 13°C, Floralife was as good as or equal to water at 2° or 7° shipping temperatures. If the flower stems were held in a plastic bag rather than dry in a box and supplied with preservative prior to and after shipping, even the 20° shipping temperature allowed as much delay to 50% leaf blackening as did dry shipping with no preservative use at 7°C.

Clonal differences were observed in the rate of leaf blackening. As much as 20% difference in the time to 50% leaf blackening was observed among clones in both water and preservative. Even when preservative slowed the rate of leaf blackening, the flower could still wilt.

Water loss from the flower varied from 25 to 50% of the water loss from a leafy stem with flower. Removal of the flower had a delaying on the development of leaf blackening. This is not at variance with a hypothesis that water loss through the flower head causes a water stress which triggers the blackening reaction.

Introduction

Discoloration of flower petals and/or foliage is regarded as a sign of poor quality in cut flowers. An extensive series of papers (c. f. review by Halevy and Mayak, 1979; Halevy, 1976, Halevy and Mayak, 1974; Mastalerz, 1953; Mayak, et al., 1974; Moe, 1975; Zieslin and Halevy, 1969; Zieslin, et al., 1978) identify research on environmental and internal components of rose petal discoloration. In one of the newer cut flower families, protea, a number of species show foliage and bract blackening (Akamine, et al., 1979; Haasbroek, et al., 1973). P r o t e a e x i m i a shows a fast rate of leaf blackening, averaging 7.1 days, while P. l o n g i f o l i a is much slower, averaging 46.1 days (Akamine, et al., 1979).

As in roses (Moe, 1975; Zieslin and Halevy, 1969) oxidative enzymes are responsible for the blackening reaction in proteas. Chief among these enzyme systems are phenyl ammonia lyase and polyphenol oxidase (unpublished results) with high activity. It was observed, however, that a stress situation was necessary to stimulate the blackening reaction. We hypothesized that water stress triggers the blackening reaction.

The normal commercial harvest practice for proteas in Hawaii is to hold the flowers dry in the field while cutting. The cut flowers are taken to packing sheds and held overnight in preservative before packing and shipping. The "Chain of Life" concept (Anon., 1978) has made some small impact, but preservatives are not widely used at the wholesale and retail levels.

Research in South Africa has demonstrated some success in suppressing blackening through the use of a preservative (Haasbroek, et al., 1973; Ireland, et al., 1967). The studies we report confirm the desirability of preservative use as well as adoption of other measures to reduce a stress situation in the cut flower.

Materials and Methods Common to All Experiments

Terminal shoots with flower buds of *Protea eximia* were cut from several clones at the Maui Agricultural Research Center at Kula, Maui, and immediately air-shipped to Honolulu where the investigations were conducted. The elapsed time from picking to initiation of an experiment was about 5 hours.

At least three stems were used for each treatment. Evaluation of the rate of leaf blackening was based on number of leaves and relative area of each leaf with darkened surface as a percentage of total leaf surface area. Observations were recorded daily until all leaves were completely black. Observations on outer bracts of the flower were made for wilting and blackening. Useful vaselife was determined as the time period from placement of the flower in water until severe wilting and blackening were evident.

Vaselife was evaluated under the following conditions: 20 to 25°C, 70 to 80% relative humidity (RH), 10 hours fluorescent light (1 watt m^{-2} day^{-1}). All solutions were prepared in deionized water, pH = 6.5 to 6.8. Floralife preservative was used at the rate of 20 g/l. Stems were recut following simulated shipping and prior to placement in solutions.

Specific details are given for individual experiments.

Results

1. Effects of simulated shipping temperatures and method of packing

After removing the basal 15 cm of foliage, the flowers were held overnight in Floralife or water, then packed dry in a box or sealed in a plastic bag and held at 2, 7, 13, or 20° for 7 days. Upon removal from these conditions, the flowers were placed in water or Floralife and evaluated for leaf blackening and flower wilting. The results (table 1) show that either 2° or 7° shipping temperatures or use of the preservative at 13° were effective in delaying leaf blackening, but flowers held dry at 20° showed blackening and some wilting before removal from simulated shipping. Sealing the flowers in a plastic bag was more effective in delaying leaf blackening and wilting than dry packing at all temperatures.

A second version of this experiment sought to determine if an ethylene absorbent packed in the plastic bag would delay the onset of leaf blackening. The comparison was made using flowers held 5 days at 7° and placed in Floralife for evaluation of the rate of leaf blackening. A difference in the rate of blackening was observed from the preceding experiment, but the inclusion of an ethylene absorbent (Purafil) appeared to enhance the rate of blackening (table 2). Foliage on stems from the sealed bag blackened at a faster rate in this experiment.

2. Effect of clone on vaselife

Initial experiments showed some differences which seemed unrelated to treatment. Since the flowers had been cut without regard to clonal differences, it was necessary to determine the effect of clone on vaselife. Flowers from 4 clones were compared for their rate of leaf blackening when held in either Floralife or water. The effect of the preservative was again very noticeable in delaying blackening (table 3) but the rate of leaf blackening differed among clones by as much as 20%.

3. Water loss from cut stems of *Protea eximia*

The flower of a protea has a relatively large surface area and may be a source of water stress through evapotranspiration. Our previous work with vegetative stems or stems with a very small bud showed little leaf blackening. Comparisons of the rate of water loss were made for intact stem and flower against defoliated stems (top 4 leaves only remaining) with a flower, and foliated stems from which the flower had been removed. The results (figure 1) showed that the flower head is capable of water loss ranging from 25 to nearly 50 percent of the water loss of the intact system. Removal of the flower head delayed by about 2 days the development of leaf discoloration to 50% blackening (table 4), while leaf removal was also effective in delaying blackening of the top leaves and outer bracts of the flower.

4. Observations

Leaf blackening can occur around injuries sustained to the leaf while the stem is still on the plant in the field. Usually the blackening is restricted to the injured area and does not enlarge. Such leaves are likely to blacken rapidly when the flower is out.

The pattern of leaf blackening usually develops from the margin of the leaf inward. The base of the leaf blackens first on the older leaves but the blackening begins closer to the tip on younger leaves. Where stems have had a preservative treatment, leaf blackening develops in a spotty pattern.

Discussion

Removal of the foliage would be a simple alternative to allowing it to become black. The protea leaf, however, is sessile and not easily removed. Also, the blackening reaction does occur on the flower bracts. Thus, a means to suppress or delay foliage blackening is essential to the marketing of this and other rapid-blackening species.

South African recommendations (Haasbroek, et al., 1973; Ireland, et al., 1967) for shipping have urged the enveloping of the flowers in plastic as well as the use of a preservative. In their experience, this has reduced blackening. We concur that these practices are desirable. Since refrigerated shipping is not always possible, the use of a plastic wrap to maintain a saturated atmosphere seems an adequate substitute if the temperature can be kept around 13° or lower.

The possible impact of ethylene in the stress reaction needs closer attention. It is not possible to exclude it as a causative factor in the leaf blackening reaction, but the faster blackening which occurred in the presence of an ethylene absorbent is a loose end.

It is clear that clonal differences exist in *Protea eximia* with respect to the rate of leaf blackening. Much of the cut protea flower production is based on seed-produced stock. Screening may enable selection of cultivars with reduced blackening potential.

Water stress in roses does contribute to bent neck (Zieslin, et al., 1978) and to failure of floret development in other cut flowers (Halevy and Mayak, 1974; Mayak and Halevy, 1971). In protea, vascular blockage apparently does not occur as readily and is apparently not associated with the leaf blackening reaction. As shown in figure 1, water uptake continues for several days after leaf blackening has reached the 100 percent mark. While it is not possible to state that adequate water uptake is occurring, it is apparent that some metabolic activity is proceeding as deteriorative (blackening) events occur. A preservative will suppress but not prevent leaf blackening.

Our best recommendations would follow those of the "chain of life," that is, place the flowers in preservative as cut in the field, seal in plastic during shipment, maintain temperatures at 13° or cooler during shipment, and use preservative following shipping. All these practices should alleviate the water stress which seems associated with the leaf blackening reaction.

References

Akamine, E. K., T. Goo, and R. Suehisa. 1979. Relationship between leaf darkening and chemical composition of leaves of species of protea. Flor. Rev. 163(4236):62-63, 107-108.

Anonymous. 1978. Chain of Life: Improving the quality and lasting attributes of roses. Flor. Rev. 162(4200):16, 57-59.

Haasbroek, F. J., G. G. Rousseau, and J. F. de Villeirs. 1973. Effect of gamma rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamarck, and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5:53-42.

Halevy, A. H. 1976. Treatments to improve water balance of cut flowers. Acta Horticulturae 64:223-230.

Halevy, A. H. and S. Mayak. 1974. Improvement of cut flower quality opening and longevity by pre-shipment treatments. Acta Horticulturae 43:335-347.

Halevy, A. H. and S. Mayak. 1979. Senescence and postharvest physiology of cut flowers, Part 1. Hort. Rev. 1:204-236.

Ireland, J. P., J. T. Meynhardt, and J. M. Strauss. 1967. When proteas become sailors. Fmg. S. Afr. 43(6):33-35.

Mastalerz, J. W. 1953. The effect of water absorption before low temperature dry storage on the development of blue color in 'Better Times' roses. Proc. Amer. Soc. Hort. Sci. 61:593-598.

Mayak, S., and A. H. Halevy. 1971. Water stress as the cause for failure of flower bud opening in iris. J. Amer. Soc. Hort. Sci. 96:482-483.

Mayak, S., A. H. Halevy, S. Sagie, A. Bar-Yoseph, and B. Bravdo. 1974. The water balance of cut roses. Physiol. Plant. 32:15-22.

Moe, R. 1975. The effect of growing temperature on keeping quality of cut roses. Acta Horticulturae 41:77-88.

Zieslin, N. and A. H. Halevy. 1969. Petal blackening in 'Baccara' roses. J. Amer. Soc. Hort. Sci. 94:629-631.

Zieslin, N., H. C. Kohl, Jr., A. M. Kofranek, and A. H. Halevy. 1978. Changes in the water status of cut roses and its relationship to bent-neck phenomenon. J. Amer. Soc. Hort. Sci. 103:176-179.

Table 3 - Days to 50% and 100% leaf blackening for 4 clones of P. e x i m i a held in deionized water or Floralife preservative.

	50% blackening	100% blackening
Water		
Clone 2	4.8	15
Clone 3	4.0	13
Clone 4	4.0	13
Clone 5	4.0	10
Floralife		
Clone 2	18	25 [a]
Clone 3	15	25 [a]
Clone 4	14	25 [a]
Clone 5	19	25 [a]

[a] At this time, 60-75% leaf blackening was evident, but the flower was severely wilted, partially dried, and partially blackened.

Table 4 - Days to 50% and 100% leaf blackening for intact, defoliated, and decapitated flower stems of P. e x i m i a held in deionized water.

	50% blackening	100% blackening
Intact stem	3.5	5.0
Defoliated stem (top 4 leaves + flower)	6.1	8.0
Decapitated	5.5	7.5

COMPUTERIZED FLORAL POSTHARVEST LITERATURE RETRIEVAL SYSTEM

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A computerized literature retrieval and storage system has been developed for postharvest-related references in floriculture, plant physiology, and other specific fields. The languages utilized are spitbol and Mark IV, but others could easily be made to work. The capabilities include retrieval by author, subject (keywords), year, and/or journal name. Typical printouts include specific listings of the asked for acticles in alphabetical order by senior author. The system can be accessed using a broad range of satellite terminals including DEC, IBM, and CRT. A procedure to print reference books directly off of the computer is being developed and should be ready by the end of 1980. Foreign articles have been translated into English by many cooperators from a number of countries, but special thanks goes to Dr. Henk de Stigter of the Netherlands. At present, approximately 4200 references are accounted for of which approximately 640 deal with roses, 400 for chrysanthemums, 560 for carnations and 210 for gladioli. Printouts can be requested by contacting the authors. At present, there is no charge for this service.

Table 1 - Number of days to 50% leaf blackening and flower wilting for P. e x i m i a flowers preconditioned in water or Floralife, held 7 days dry in boxes or in plastic bags, and evaluated in water or Floralife

Precondition	Shipping	Evaluation	Days to 50% leaf blackening: shipping temperatures (°C) 2	7	13	20
Water	Dry	Water	4	3	0	-4 [a]
Floralife	Dry	Floralife	7	9	5	-4 [a]
Floralife	Bag	Floralife	8	10	7	3
			Days to flower wilting: shipping temperatures (°C) 2	7	13	20
Water	Dry	Floralife	5	3	2	-3 [a]
Floralife	Dry	Floralife	5	4	3	-3 [a]
Floralife	Bag	Floralife	7	8	5	5

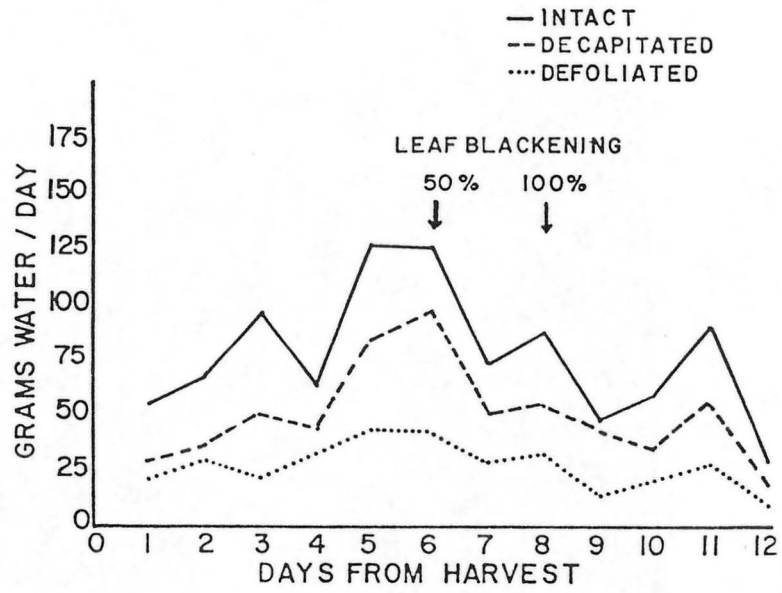
[a] Leaf blackening and wilting were evident before removal from dry storage.

Table 2 - Percentage leaf blackening with time following removal from 5 days of simulated shipping at 7°C. Evaluation in Floralife (20 g/l).

Storage condition	Days after removal from storage: 0	2	4	6	10	15
Dry in box	0	7	10	12.5	15	25
Sealed in plastic bag	0	8	13	25	25	52
Sealed in plastic bag plus C_2H_4 absorbent	10	18	45	57	65	70
No storage; held continuously in preservative [a]	48	93	98	100	100	100

[a] Add 5 days to column headings.

Figure 1. Grams water loss per day for intact, defoliated, and decapitated flower stems of P. e x i m i a held in deionized water.



getative body. To realise that, a rapid increase in light-
ea is required, either through rapid initiation of leaves
ormation of large leaves (Langton and Cockshull, 1976).
unwanted, however, as they require wider spacing. Thus
ds to be on rapid initiation of small leaves. Screening for
is under way.

79. Effects of irradiance and temperature on flowering of *Chrysan-
um* Ramat. in continuous light. Ann. Bot., 44: 451–560.
Hughes, A.P., 1972. Flower formation in *Chrysanthemum mori-
nce of light level. J. Hortic. Sci., 47: 113–127.
and, D.W. and Langton, F.A., 1981. The effects of day and night
flower initiation and development in Chrysanthemum. Acta Hortic.,

Selection for wide temperature adaptation in *Chrysanthemum mori-*
Hemsl. Neth. J. Agric. Sci., 26: 110–118.
Genetic analysis in *Chrysanthemum morifolium*. I. Flowering time
er at low and optimum temperature. Euphytica, 33: 455–463.
Cockshull, K.E., 1976. An ideotype of chrysanthemum (*C. morifolium*
ortic., 63: 165–175.
g, J. and Smeets, L., 1984. Effect of day and night temperatures
otoperiods on growth and flowering of *Chrysanthemum morifolium*
Hortic., 22: 373–381.
33. Zur Karyogenetik der Gattung Chrysanthemum. J. Sci. Hiroshima
v. 2, 1: 1–98.

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SO: Sci-Hortic. Amsterdam : Elsevier Science Publishers. Apr 1986. v. 28 (3) p. 271-279. ill.

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POST-HARVEST PHYSIOLOGY OF WARATAH INFLORESCENCES (*TELOPEA SPECIOSISSIMA*, PROTEACEAE)

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ABSTRACT

Faragher, J.D., 1986. Post-harvest physiology of waratah inflorescences (*Telopea speciosissima*, Proteaceae). *Scientia Hortic.*, 28: 271–279.

The post-harvest development and senescence of cut waratah inflorescences (*Telopea speciosissima* R.Br.) held at 20°C is described. Individual flowers of the raceme opened and after 4 or more days the perianth abscised, wilted and changed color from bright red to blue-red. The end of vase life was defined as a readily discernible wilting and blueing of either flowers or of the bracts which subtend the inflorescence. During vase life the fresh weight, water content and anthocyanin concentration of both flowers and bracts decreased. Water uptake by the whole inflorescence decreased, the cumulative nett water loss increased and the fresh weight of the inflorescence decreased. Ethylene production by flowers increased to a maximum and then declined, while the low ethylene production by bracts slowly decreased. Vase life was extended from 7.9 to 11.7 days when the vase water was treated with chlorine bactericide (sodium dichloroisocyanurate, 25 mg l^{-1} available Cl). This increased vase life was accompanied by increased water uptake, decreased nett water loss, and a slower rate of decrease in flower and bract fresh weight and water content. Vase life was also extended by early harvest, when 6% of individual flowers were open rather than 48%. It was concluded that decreased water uptake and flower abscission were the major physiological factors which limited vase life.

Keywords: flower abscission; flower senescence; protea; vase life.

INTRODUCTION

Waratah (*Telopea speciosissima*, R.Br. Proteacae) is a new cut-flower crop, and with its spectacular red inflorescence has a potentially large market (Fig. 1). To develop suitable post-harvest handling techniques for this flower, some knowledge of its post-harvest characteristics and physiology is necessary. There is only one preliminary report on this subject to date, in which it was stated that the optimum stage of harvest was when 0–5% of individual flowers had opened, that vase life at 20°C was 13 days, and that none of a wide range of floral preservatives improved the vase life (Worrall, 1983).

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Fig. 1. Waratah inflorescence showing the raceme of flowers, the surrounding bracts, leaves and stem. Magnification × 0.4

While it is expected that the post-harvest physiology of waratahs would be similar to that of *Protea* sp., with the exception of work on leaf blackening there is little published information on this subject (Jacobs, 1981).

The aim of this work was to investigate and describe the basic post-harvest characteristics and physiology of waratah flowers. Flower development, ageing, and senescence are described in terms of: flower opening; perianth abscission; changes in fresh weight, water content, anthocyanin content, and ethylene production of flowers and bracts; and water uptake and transpiration by the inflorescence. The relationship between these changes and the ultimate vase life of the inflorescence was investigated further by applying a treatment which increased water uptake and extended vase life.

MATERIALS AND METHODS

Flowers. — The waratah inflorescence is a terminal raceme of 150—200 red flowers surrounded by red bracts (Fig. 1 and Payne, 1982). Inflorescences were cut during October 1984 from 20-year-old seedling plants growing 50 km east of Melbourne. Inflorescences were cut at different stages of development, as measured by the percentage of flowers which had opened. In one experiment these stages were 6, 27 and 48% open, and for other experiments ranged between 25 and 33% open, the latter being the stage of development of commercially cut flowers. The stems were placed in water and transported to the laboratory within 1 h. Before use, stems were re-cut to 30 cm length and the lower one-third of the leaves was cut off. In the following work the

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term inflorescence refers to this material, which included the true inflorescence, leaves and stem. All inflorescences were dipped in Rovral fungicide (iprodione, May and Baker, Australia) at 1 g l^{-1} for 30 s. Each experiment was carried out with 7 or 8 replicate inflorescences from a bulk sample taken from 40 plants.

Experimental treatments. — The inflorescences were held with stems in water at 20 ± 1°C and 60% RH, with continuous white fluorescent light (Osram, Australia) of 5 W m^{-2} irradiance. In one experiment a chlorine bactericide was used (Halevy and Mayak, 1981), and sodium dichloroisocyanurate (Filtrite, stabilized chlorine, Clark Rubber, Australia) was added to the vase water at 25 mg l^{-1} available chlorine. The vase water, and chlorinated water, were changed every second day.

Measurements. — Flower opening was expressed as the percentage of total flowers on the raceme which had opened; that is, in which the style had emerged. Flower abscission was expressed as the percentage of total flowers in which the perianth was visibly separated from the pedicel after the flowers were gently brushed with a finger. The end of vase life was defined as when there was readily discernible wilting or blueing of at least one-third of the flowers, or all of the bracts.

Fresh weight, dry weight, water content, anthocyanin concentration and ethylene production were measured on a representative bract and the outermost (oldest) flower from each of 6—10 replicate inflorescences. Dry weight was measured after drying for 48 h at 95°C. Water content (g H_2O/g dry weight) is defined as: (fresh weight − dry weight) ÷ dry weight (Slatyer, 1967). Anthocyanin was measured after extracting the pigment from 1.54 cm^2 of bract, or one whole flower, in 20 and 40 ml, respectively, of 1% HCl in 49% methanol, 50% water. The extract absorbance was measured at 530 nm, the wavelength of maximum absorbance in all samples. Ethylene production (evolution) rates were measured by sealing bracts or flowers in 55-ml test tubes for 4—6 h at 20°C and then the ethylene concentration in a 1-ml sample of the head-space gas was measured by gas chromatography, as described by McGlasson (1969).

Water uptake and transpiration of the whole inflorescence was measured as follows. The inflorescence was held in a 1-l bottle and the narrow mouth of the bottle was sealed with plastic film, which effectively prevented water evaporating out of the mouth of the bottle. At each measurement time, the following measurements were made:

(i) weight of bottle, water and inflorescence;
(ii) weight of bottle and water only.

Water uptake between measurement times was the difference in values of measurement (ii) between the two times. Transpiration was the difference in values of measurement (i) between the two times. Nett water loss from the inflorescence was calculated as transpiration minus water uptake.

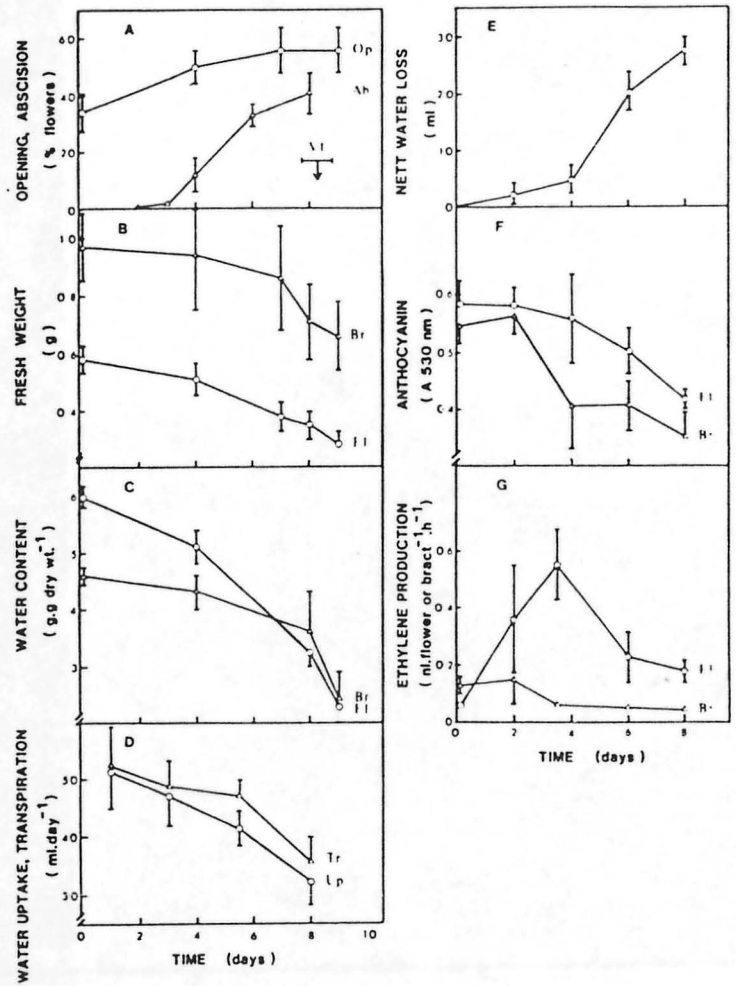


Fig. 2. Changes in inflorescence, flowers and bracts of cut waratahs during vase life. A. Flower opening (○, Op) and perianth abscission (△, Ab). Vase life (Vl) is indicated by the arrow. B and C. Fresh weight and water content of bracts (△, Br) and the outermost flowers (○, Fl). D. Water uptake (○, Up) and transpiration (△, Tr) by the inflorescence. E. Nett water loss (cumulative) from the inflorescence. F. and G. Anthocyanin levels and ethylene production rates of flowers (○, Fl) and bracts (△, Br). Values are means of 8 replicates ± standard errors.

RESULTS

Development of the cut inflorescence. — The individual flowers mature from the base to the apex of the inflorescence, the perianth opens and recurves and the style emerges. Flowers continued to open on the cut inflorescence, but reached a maximum (with less than 100% flowers open) before the end of vase life (Fig. 2A). After 4 days at 20°C, the perianth of the oldest flowers abscised from the pedicel and the number of abscised flowers increased with time (Fig. 2A). The abscised perianths wilted and changed color from bright red to blue-red. Later, the unabscised flowers and the bracts slowly wilted and changed color from bright red to blue-red.

Vase life. — The characteristics which determined the end of vase life were considered to be, firstly, an excessive number (one-third) of wilted, faded and bluish flowers, and secondly, the appearance of bracts when they wilted, faded and turned bluer and then brown. Generally the flowers were unacceptable before the bracts, and usually the unacceptable flowers were those which had abscised. The leaves remained acceptable. The mean vase life of typical flowers was 8.3 days (Fig. 2A).

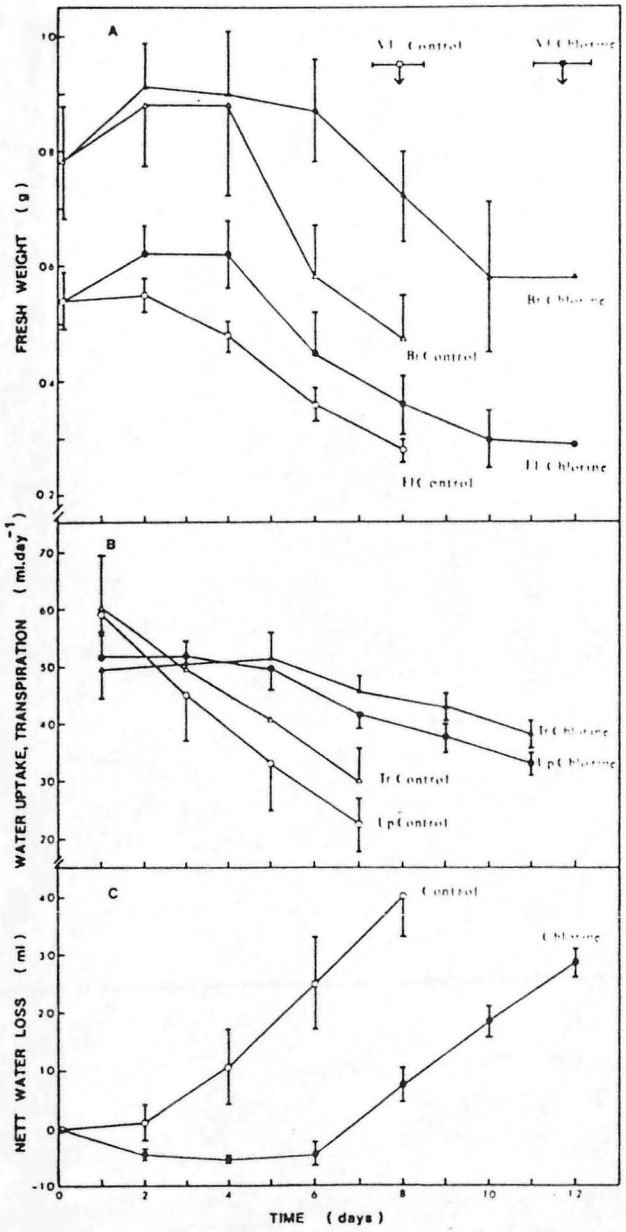
Effects of harvest stage. — When the inflorescence was harvested at progressively later stages of development, with more flowers open, the subsequent vase life was slightly reduced and the maximum number of flowers which opened during the vase life increased (Table I). Although the effect of harvest stage on vase life was not statistically significant when the three stages were compared using ANOVA and Duncan's multiple range test, the vase life of Stage C was significantly less than that of Stage A when just these two were compared by *t*-test (Table I).

Changes in the inflorescence during vase life. — The fresh weight of the oldest flowers and of bracts decreased with age, particularly near the end of vase life (Fig. 2B). In flowers, the rate of weight loss increased after

TABLE I

Effect of harvest stage on vase life and flower opening. Inflorescences were harvested at three stages, each with a different percentage of the individual flowers open. The vase life at 20°C and the percentage of open flowers at the end of vase life were measured. Values are means ± standard errors of 7 replicates; values in a column followed by a different letter are significantly different ($P < 0.05$, Duncan's multiple range test)

Harvest stage (% flowers open)	Vase life (days)	Flower opening (%)
A 6 a	10.3 ± 0.6 a	45 a
B 27 b	9.3 ± 0.5 a	60 b
C 48 c	8.9 ± 0.6 a	72 c



abscission, and in fact 70% of the weight loss in flowers during vase life was the result of weight loss in the perianth (data not shown). Water content of flowers and bracts also decreased with age (Fig. 2C). In flowers, water content decreased faster than fresh weight, and after 4 days it was significantly less than at harvest. The dry weight of bracts and flowers did not change during vase life (data not shown).

Water uptake by the inflorescence decreased with time (Fig. 2D). Transpiration also decreased, and although it was always slightly greater than uptake, this difference was not statistically significant due to variability between inflorescences (Fig. 2D). However, the cumulative difference between water uptake and transpiration, i.e. nett water loss, increased with time at 20°C (Fig. 2E) and this led to a significant decrease in inflorescence weight; from 144 to 116 g ($P < 0.01$).

Anthocyanin concentration in bracts and in the oldest flowers decreased with time, and at the end of vase life was approximately 70% of that at harvest (Fig. 2F). As the inflorescence aged, flowers and bracts appeared to be more blue. However, there was no measurable change in the wavelength of maximum absorbance of the anthocyanin extracts (data not shown).

Ethylene production (evolution) by flowers increased with age and then decreased (Fig. 2G). Ethylene production by bracts only decreased slowly with age (Fig. 2G). The maximum rate of 0.5 nl flower^{-1} h^{-1} is equivalent to approximately 1 nl g^{-1} h^{-1} and the rate of 0.1 nl bract^{-1} h^{-1} is equivalent to approximately 0.1 nl g^{-1} h^{-1}.

Changes associated with extended vase life. — The vase life of waratahs increased from 7.9 to 11.7 days when the vase water was treated with the chlorine bactericide/germicide sodium dichloroisocyanurate (Fig. 3A). The longer life was associated with a slower rate of decrease in bract and flower fresh weights (Fig. 3A) and water contents (data not shown). Chlorine treatment greatly reduced the rate of decrease in water uptake, so that uptake was greater than in controls (Fig. 3B). Further, chlorine greatly reduced nett water loss from the inflorescence and delayed the time when water loss was greater than zero (Fig. 3C). Chloride treatment did not affect flower opening, anthocyanin levels, or ethylene production (data not shown).

DISCUSSION

The changes in the cut waratah inflorescence which determined the end of vase life were wilting, loss of bright red color, and appearance of blue-red

Fig. 3. Effects of chlorine bactericide in vase water on cut waratahs during vase life. Chlorine was provided by adding sodium dichloroisocyanurate at 25 mg l^{-1} available chlorine. A. Fresh weight of bracts (△ ▲, Br) and outermost flowers (○ ●, Fl) and inflorescence vase life (Vl). B. Water uptake (○ ●, Up) and transpiration (△ ▲, Tr). C. Nett water loss (cumulative) from the inflorescence. Values are means of 8 replicates ± standard errors.

colors. Similar changes occurred during senescence of inflorescences which remained on the plant. The measured characteristics which were most closely associated with the visible end of vase life were decreases in fresh weight and water content of flowers and bracts. Anthocyanin content was not so clearly related to vase life, since chlorine treatment, which increased vase life, did not affect anthocyanin levels. The vase life of 8—10 days was less than the 13 days at 20°C reported by Worrall (1983) under very similar environmental conditions. The reasons for this difference could be the more mature stage at which my flowers were cut and differences in seedlings and growing conditions. However, the 8—10-day vase life does indicate that waratahs do not necessarily have an exceptionally long life. The effect of harvest stage on vase life was small, but the 1.4-day difference between flowers cut with 6 and 48% of flowers open may be commercially important.

The observed decreases in fresh weight and water content of flowers and bracts and the consequent wilting, blueing and end of vase life may be caused by one or more of the following changes in the inflorescence: (i) decreased water uptake and increased nett water loss; (ii) perianth abscission; (iii) increased ethylene production. Presumably, decreased water uptake and increased nett water loss are, at least in part, responsible for decreases in fresh weight and water content. The fact that a treatment (chlorine) which increased water uptake increased the subsequent vase life strongly suggests that vase life depends on water uptake. The primary action of chlorine, a bactericide/germicide (Halevy and Mayak, 1981), appears to have been to slow down the rate of decrease in water uptake which occurred during vase life (Fig. 3B). This suggests that microorganisms or their products caused, at least in part, the decrease in water uptake. In flowers, the decreases in fresh weight and water content were accompanied by perianth abscission, and the first flowers which were visibly wilted and blue were those in which abscission had occurred. Thus, abscission of the perianth is a major cause of the end of vase life. The cause of abscission has not yet been investigated, but common causes of flower abscission are ethylene action, pollination, water stress and other hormonal changes (Halevy and Mayak, 1981). There was an increase in ethylene production and a decrease in water content in waratah flowers before abscission, so these results are consistent with either ethylene or water stress causing abscission. However, in inflorescences which remained on the plant, abscission occurred before there was any visible wilting, and this suggests that abscission does not necessarily depend on water stress. Pollen is present on the style, adjacent to the stigma, when the flower opens, so pollination in the laboratory is possible. The causes of abscission will be investigated in future work.

In several flowers an increase in ethylene production, as observed in waratah, precedes ethylene-induced flower senescence (Halevy and Mayak, 1981). Although it is possible that ethylene has such a role in waratah, the results which showed that chlorine treatment extended vase life without

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delaying the climacteric rise in ethylene production suggest that ethylene is probably not a critical factor in senescence. The role of ethylene in waratah flower senescence will be investigated in future experiments.

These experiments have indicated several points which will have to be taken into account when post-harvest handling techniques are developed for waratahs: the possible advantages of harvest at an early stage of development; the importance of water relations; and the problem of flower abscission. One technique to improve water balance, i.e. repeated addition of slow-release chlorine bactericide/germicide to water, has been demonstrated here.

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REFERENCES

Halevy, A.H. and Mayak, S., 1981. Senescence and post-harvest physiology of cut flowers. Part 2. Hortic. Rev., 3: 59—143.

Jacobs, G., 1981. Post-harvest handling of proteas. In: P. Mathews (Editor), Growing and Marketing of Proteas, Vol. 1. Proteaflora Enterprises, Melbourne, pp. 40—51.

McGlasson, W.B., 1969. Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. Aust. J. Biol. Sci., 22: 489—491.

Payne, W.H. (Editor), 1982. Aust. Plants. 11: 333.

Slatyer, R.O., 1967. Plant—Water Relationships. Academic Press, London, p. 150.

Worrall, R.J., 1983. Growing waratahs commercially. Aust. Hortic., 81: 101—103.

Potassium and phosphate absorption by excised ordinary and proteoid roots of the Proteaceae

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Potassium and phosphate absorption by excised ordinary and proteoid roots of certain species of the Proteaceae was investigated, using ^{86}Rb (as substitute for ^{42}K) and ^{32}P as tracers. The respiratory uncoupler, 2,4-dinitrophenol (DNP), inhibited the uptake of potassium and phosphate. The greater inhibition obtained with proteoid roots possibly suggests that they possess a greater capacity for metabolic absorption than the ordinary roots. Absorption of potassium and phosphate over a concentration range of 0,005 – 50 mmol dm^{-3} KCl and KH_2PO_4 respectively, was investigated. Lineweaver-Burk kinetic analysis of the data on potassium absorption in the low concentration range (0,005 – 1,0 mmol dm^{-3} KCl) revealed a more effective absorption mechanism in the case of proteoid roots. Kinetic analysis of the data on phosphate absorption in both the low (0,02 – 1,0 mmol dm^{-3} KH_2PO_4) and high (1,0 – 50 mmol dm^{-3} KH_2PO_4) concentration ranges points to a more effective absorption mechanism in proteoid roots. Phosphate absorption by proteoid roots showed a distinct peak between pH 4 and 5,5.
S. Afr. J. Bot. 1986, 52: 277 – 281

Opname van kalium en fosfaat deur afgesnyde gewone en proteoïede wortels van sekere soorte van die Proteaceae is ondersoek. As merkers is ^{86}Rb (as plaasvervanger vir ^{42}K) en ^{32}P gebruik. Die respiratoriese ontkoppelaar, 2,4-dinitrofenol (DNP) het die opname van kalium en fosfaat deur gewone en proteoïede wortels onderdruk. Die groter mate van onderdrukking by proteoïede wortels dui moontlik daarop dat hulle oor 'n groter vermoë tot metaboliese opname as die gewone wortels beskik. Die opname van kalium en fosfaat oor 'n konsentrasiegebied van 0,005 – 50 mmol dm^{-3} KCl en KH_2PO_4 onderskeidelik, is ondersoek. Lineweaver-Burk kinetiese analise van die gegewens ten opsigte van kaliumopname in die lae konsentrasiegebied (0,005 – 1,0 mmol dm^{-3} KCl), dui op 'n meer effektiewe meganisme van opname by proteoïede as gewone wortels. Kinetiese analise van die resultate verkry tydens fosfaatopname in sowel die lae (0,02 – 1,0 mmol dm^{-3} KH_2PO_4) as die hoë (1,0 – 50 mmol dm^{-3} KH_2PO_4) konsentrasiegebied, dui ongetwyfeld op 'n meer effektiewe meganisme van metaboliese opname by proteoïede wortels. Fosfaatopname deur proteoïede wortels het 'n duidelike piek tussen pH 4 en 5,5 getoon.
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Keywords: Phosphate, potassium, proteoid roots

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Introduction

Proteoid roots are characteristic of almost all species of the Proteaceae. Lamont (1980) determined that proteoid roots were present in 50 species which can be regarded as representative of the 13 genera of the Proteaceae in southern Africa.

The term 'proteoid roots' was initially used only to describe the dense clusters of fine roots which occur in longitudinal rows along the ordinary roots of members of the Proteaceae. According to Gardner *et al.* (1982) similar structures have also been found on species of other plants, e.g. *Lupinus*.

According to Lamont (1972), proteoid roots can be distinguished from ordinary roots by their relatively uniform length, their restricted growth, the great abundance of root hairs present, their inability to form new roots and their restricted lifetime — approximately two to three months in the case of *Leucospermum parile* (Jongens-Roberts 1981).

It is further generally accepted that proteoid roots are specialized structures which developed in reaction to the extremely low concentrations of nutrients present in the soils in which these plants are normally found. Groves (cited by Jeffrey 1967) determined that proteoid root development in *Banksia ornata* occurred at an earlier stage in culture solutions with the lowest phosphate content. Establishment of young seedlings, especially on poor substrates, is apparently made possible by a high nutrient status of the seed (Groves & Keraitis 1976).

The fact that most members of the Proteaceae can survive and even flourish on poor substrates, and that proteoid roots are such a characteristic component of their root systems, might be an indication that proteoid roots fulfil some important role in their mineral nutrition.

In this investigation an attempt was made to determine the possible role of proteoid roots in the mineral nutrition of the Proteaceae by comparing the ion absorption capacity of excised ordinary and proteoid roots.

Materials and Methods

Plants, 8 to 18 months old, of those species indicated in the relevant tables and figures, were obtained from nurseries.

The roots were washed in running tap water, followed by rinsing in deionized water. The young root tips and the clusters of proteoid roots were excised and suspended in an aerated 0,5 mmol dm^{-3} $CaSO_4$ solution (Epstein 1961). Following determination of their fresh mass, root samples were placed in nylon gauze bags (50 × 70 mm), consisting of two compartments (Jooste & De Bruyn 1979). Ordinary roots were placed in one compartment and proteoid roots in the other. This ensured that the two types of roots were subjected to

identical experimental conditions.

The bags containing the root samples were placed in an aerated 0,5 mmol dm^{-3} $CaSO_4$ solution at 25°C. The minimum volume of this solution — the so-called 'intermediate solution' — was 150 cm^3 per sample.

After 30 min in the intermediate solution, the samples were transferred to the various experimental solutions, which were also continuously aerated. Unless otherwise specified, the samples were exposed for 1 h to the experimental solution at 25°C.

Each sample was thereupon rinsed for a total of 1 min in a separate series of four beakers each containing 200 cm^3 deionized water.

In most cases (see text), the samples were subjected to a desorption treatment at 2°C for 30 min in a continuously aerated desorption medium with minimum volume of 250 cm^{-3} per sample. The experimental solutions and desorption media contained KCl or KH_2PO_4 at a concentration of 0,5 mmol dm^{-3} (unless otherwise specified) in a 0,5 mmol dm^{-3} $CaSO_4$ solution. To the experimental solutions approximately 333 kBq ^{86}Rb or ^{32}P per 2 dm^3 were added as tracers. ^{86}Rb was used as substitute for ^{42}K (Epstein & Hagen 1952; Epstein 1961; Rains *et al.* 1964) and both isotopes were obtained from the Radiochemical Centre, Amersham, U.K.

Unless otherwise specified, the pH of both the experimental solution and desorption medium varied between 5,5 and 6,0. Where the effect of 2,4-dinitrophenol (DNP) was studied, the latter was used at a concentration of 0,5 mmol dm^{-3}.

Following removal of the samples from the experimental solution or the desorption medium, they were placed on absorbent paper for 12 h to dry before ashing. (Too dry material is brittle and difficult to remove from the bags.)

The samples were dry-ashed according to an adaptation of the method described by Du Preez *et al.* (1981) and analysed radiometrically by liquid scintillation counting using a commercial scintillation mixture. Potassium and phosphate uptake were calculated from the ^{86}Rb and ^{32}P content of the samples and the specific activity of the experimental solutions.

Two to three replicates of each treatment were employed; each experiment was repeated at least twice on consecutive days. The mean and standard error for each treatment were calculated. Differences between means of more than twice the standard error were regarded as significant.

Results and Discussion

Insufficient quantities of suitable experimental material (plants of the same age with proteoid roots) necessitated the use of more than one species. However it was assumed that the role and behaviour of roots, especially proteoid roots, would be the same in all species.

Short term absorption studies of this nature are usually carried out with 'low salt' roots (roots with a low content of ions in general, and of the ion under investigation in particular). Since it is difficult (or even impossible) to generate low salt roots of the Proteaceae, the ion content of ordinary and proteoid roots of *Protea repens* growing in the field, was therefore determined during a period of five months (Table 1).

No definite pattern concerning differences in ion content between the two types of roots was evident. Interpretation of the results took into account the possible effect of initial differences in potassium and phosphate content of the two root types.

The effect of DNP on potassium and phosphate absorption

In investigating the metabolic nature of absorption of mineral elements by plant tissue, metabolic inhibitors are generally employed (Ordin & Jacobson 1955; Sutcliffe & Baker 1974;

Table 1 Ion content (± standard error) of roots of *Protea repens* growing in the field. Average of 5 replicates. o. roots – ordinary roots; p. roots – proteoid roots

	Ion content (mg g^{-1})							
	P		K		Ca		Na	
Date	o. roots	p. roots	o. roots	p. roots	o. roots	p. roots	o. roots	p. roots
15/5	0	0,59 ± 0,00	3,00 ± 0,08	1,37 ± 0,08	8,34 ± 2,14	8,69 ± 0,36	3,32 ± 0,01	1,29 ± 0,00
15/6	0	0	1,88 ± 0,00	1,87 ± 0,34	8,63 ± 1,68	5,85 ± 0,08	3,02 ± 0,01	2,06 ± 0,15
15/7	0,81 ± 0,00	0,55 ± 0,00	2,44 ± 0,09	1,79 ± 0,02	6,16 ± 0,84	5,12 ± 1,24	1,94 ± 0,04	1,16 ± 0,01
30/7	0,70 ± 0,02	1,14 ± 0,10	2,53 ± 0,21	3,88 ± 0,08	5,76 ± 0,21	4,67 ± 0,05	1,88 ± 0,08	1,87 ± 0,11
15/8	0,33 ± 0,00	0,59 ± 0,00	2,36 ± 0,02	2,64 ± 0,01	7,22 ± 0,09	6,64 ± 0,27	2,12 ± 0,02	2,38 ± 0,05
30/8	0,87 ± 0,01	0,40 ± 0,00	2,63 ± 0,00	1,45 ± 0,00	6,68 ± 0,06	5,28 ± 0,13	2,39 ± 0,21	1,64 ± 0,01
15/9	0,73 ± 0,02	0,38 ± 0,00	2,00 ± 0,00	1,01 ± 0,00	7,00 ± 0,46	5,01 ± 0,21	2,33 ± 0,01	1,66 ± 0,00
30/9	0,36 ± 0,01	0,24 ± 0,00	2,55 ± 0,01	1,33 ± 0,00	8,33 ± 0,78	5,33 ± 0,11	2,00 ± 0,00	1,33 ± 0,00
15/10	0,07 ± 0,01	0,33 ± 0,02	2,22 ± 0,02	2,22 ± 0,14	5,21 ± 0,00	5,90 ± 0,12	2,22 ± 0,02	2,50 ± 0,00

Jooste 1973). In this investigation the respiratory uncoupler, DNP, was used.

In the study of both potassium and phosphate absorption, two experimental solutions were used; one with and the other without DNP. Two absorption periods were employed, namely 15 and 60 min. Half of the samples in each of the treatments were subjected to a desorption treatment.

Tables 2 and 3 show that absorption of both potassium and phosphate was suppressed by DNP, and that this inhibition was greater in proteoid than in ordinary roots. This was particularly striking in the case of the 15 min absorption period.

It is also clear that desorption was responsible for the removal of a considerable fraction of the absorbed potassium and phosphate in both ordinary and proteoid roots. No definite conclusions can therefore be drawn concerning any difference in the extent of the apparent free space in the two types of roots.

Potassium and phosphate absorption at different concentrations

Epstein *et al.* (1963) proposed two mechanisms of ion absorption — one operating at a concentration of the ion in the external solution of up to 1 mmol dm^{-3} (mechanism 1), and the other from 1 mmol dm^{-3} upwards (mechanism 2). Different interpretations are currently given to this finding, for example by Nissen (1971) and Gerson & Poole (1971). Nevertheless, absorption studies over a wide concentration range supply valuable information concerning the ability of different plant roots to absorb ions.

Jackman (1965) studied the absorption of rubidium by excised roots of two grass species and two legumes. The above-mentioned two absorption mechanisms were observed throughout. It was further established that the maximum rate of absorption (V_{max}) of mechanism 1 was much greater for grass roots than for the roots of the legumes. The corresponding values for mechanism 2 were, on the other hand, found to be very similar. Jackman suggests that these findings agree with the performance of these species in the field. Legumes respond well to applied potassium when grown with grasses, but where deficiency conditions exist, when only mechanism 1 is operating, the legumes appear to compete less successfully with the grasses.

In the light of the above-mentioned findings, and in view of the fact that Proteaceae generally grow in relatively poor soils, the uptake of potassium and phosphate in the 'low' and 'high' concentration ranges was compared.

Potassium and phosphate absorption was studied over a concentration range of 0,005 – 50 mmol dm^{-3} KCl and KH_2PO_4 respectively; 0 – 1 mmol dm^{-3} representing the low, and 1 – 50 mmol dm^{-3} the high concentration range.

An absorption period of 1 h was followed by a desorption treatment of 30 min. The results are presented in the form of a Lineweaver-Burk transformation of the Michaelis-Menten

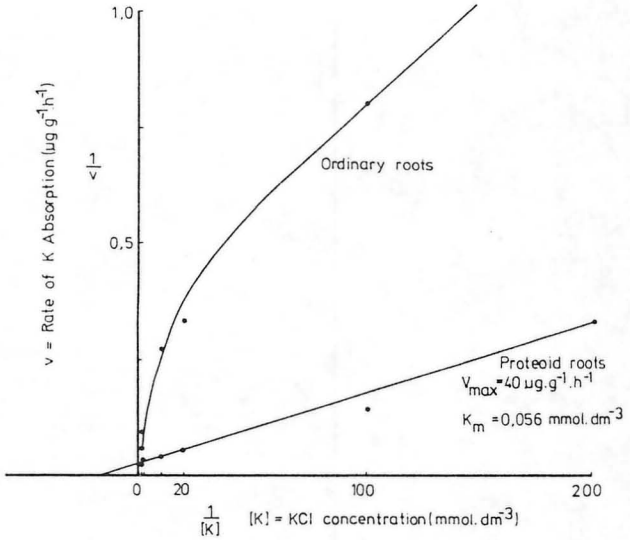

Figure 1 Lineweaver-Burk plot of rate of potassium absorption by roots of *Protea eximia* (Salisb. ex Knight) Fourcade in the low concentration range (0,005 – 1 mmol dm^{-3}).

Table 2 Effect of 2,4-dinitrophenol (DNP) on potassium absorption (± standard error) by roots of *Leucospermum cordifolium* (Salisb. ex Knight) Fourcade

	K uptake (µg g^{-1} sample)							
	Proteoid roots				Ordinary roots			
	with desorption		no desorption		with desorption		no desorption	
Absorption period (min)	KCl	KCl + DNP	KCl	KCl + DNP	KCl	KCl + DNP	KCl	KCl + DNP
15	2,34 ± 0,31	1,12 ± 0,13	5,59 ± 0,81	2,60 ± 0,32	1,95 ± 0,09	1,62 ± 0,09	4,90 ± 0,12	3,64 ± 0,54
60	7,07 ± 0,10	2,94 ± 0,19	14,82 ± 0,32	4,97 ± 0,66	6,04 ± 0,21	3,18 ± 0,67	8,07 ± 0,22	5,38 ± 0,06

Table 3 Effect of 2,4-dinitrophenol (DNP) on phosphate absorption (± standard error) by roots of *Protea repens* (L.) L.

	P uptake (µg g^{-1} sample)							
	Proteoid roots				Ordinary roots			
	with desorption		no desorption		with desorption		no desorption	
Absorption period (min)	KH_2PO_4	KH_2PO_4 + DNP	KH_2PO_4	KH_2PO_4 + DNP	KH_2PO_4	KH_2PO_4 + DNP	KH_2PO_4	KH_2PO_4 + DNP
15	14,43 ± 1,24	1,45 ± 0,32	22,9 ± 1,76	2,79 ± 0,38	13,34 ± 0,58	5,30 ± 0,58	20,08 ± 0,88	9,59 ± 1,06
60	51,97 ± 5,63	3,61 ± 0,40	66,86 ± 0,45	5,31 ± 0,87	36,50 ± 0,59	8,81 ± 0,64	45,42 ± 1,99	13,89 ± 0,73

equation. The calculated values for V_{max} and K_m are also shown.

In the case of potassium absorption in the low concentration range (Figure 1), a straight-line relationship was obtained with proteoid roots, but not with ordinary roots. The latter might indicate that absorption by ordinary roots is not controlled by metabolic processes to such a great extent as in the case of the proteoid roots. The values for V_{max} and K_m with proteoid roots were 40 µg K g^{-1} h^{-1} and 0,056 mmol dm^{-3} KCl respectively. This shows that the mechanism for potassium absorption functions quite effectively in these roots.

In the high concentration range, more potassium was absorbed by the proteoid roots than by the ordinary roots. Application of the Lineweaver-Burk kinetic analysis to these data was, however, not successful, and is therefore not included. This is not unexpected, because according to Epstein (1972) this is usually not possible in the high concentration range. Similar effects were obtained by Jooste & De Bruyn (1979) in the case of iron absorption by excised root tips and leaf discs of the bean plant (*Phaseolus vulgaris* L.)

On the other hand, kinetic analysis of the results in respect of phosphate absorption in both the low and high concentration ranges displayed a straight-line relationship (Figures 2 & 3).

In the low concentration range (Figure 2) the values for K_m and V_{max} with proteoid roots were 0,074 mmol dm^{-3} KH_2PO_4 and 35,71 µg P g^{-1} h^{-1} respectively. The corresponding values with ordinary roots were 0,103 mmol dm^{-3} KH_2PO_4 and 27,78 µg P g^{-1} h^{-1} respectively. The lower K_m value and higher V_{max} value obtained with proteoid roots are indications of a more effective mechanism of metabolic absorption than that operating in ordinary roots.

Relatively high K_m values were obtained with both proteoid and ordinary roots in the high concentration range (Figure 3), indicating a lower affinity between the ion and carrier than in the low concentration range. Nevertheless, the K_m value with proteoid roots (5,076 mmol dm^{-3}) was considerably lower than with ordinary roots (45,45 mmol dm^{-3}). However, V_{max} values were in this case higher with ordinary roots (344,83 µg P g^{-1} h^{-1}) than with proteoid roots (222,22 µg P g^{-1} h^{-1}).

These results seem to indicate that proteoid roots, especially in the low concentration range, possess a highly effective mechanism of metabolic absorption. In the case of anion absorption, it even exists in the high concentration range.

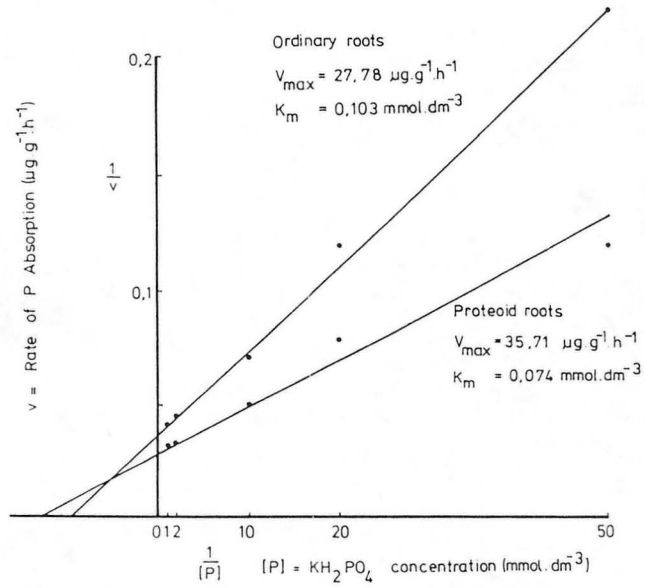

Figure 2 Lineweaver-Burk plot of rate of phosphate absorption by roots of *Protea compacta* R.Br. in the low concentration range (0,02 – 1 mmol dm^{-3}).

Potassium and phosphate absorption at different pH values

Since the pH of the soil in which the various species of the Proteaceae grow is relatively low, of the order of pH 4,0 – 5,5 (Walters 1980), potassium and phosphate absorption by ordinary and proteoid roots were compared over a pH range of approximately 3 to 8.

The experimental solutions contained potassium and phosphate at a concentration of 0,5 mmol dm^{-3} and the pH was adjusted with 0,1 N HCl or 0,1 N NaOH (Epstein 1955; Rains *et al.* 1964). Following removal from the experimental solutions, the samples were subjected to a desorption treatment for 30 min.

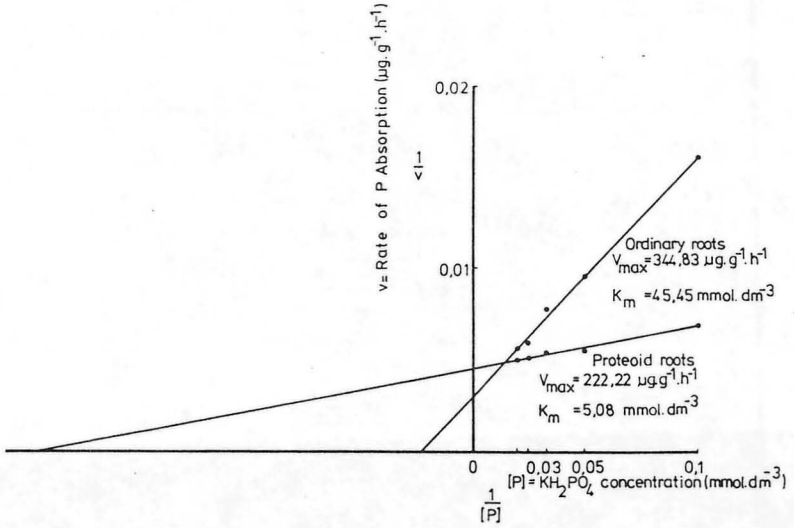

Figure 3 Lineweaver-Burk plot of rate of phosphate absorption by roots of *Protea compacta* R.Br. in the high concentration range (1 – 50 mmol dm^{-3}).

Figure 4 shows the results on potassium absorption. At pH 3, absorption by proteoid and ordinary roots did not differ. With increasing pH, potassium absorption by both root types increased, but the increase was much greater with the proteoid roots. Above pH 5, absorption by the ordinary roots declined whereas that by proteoid roots remained more or less constant.

In the case of phosphate absorption (Figure 5) the same tendency was observed with ordinary roots, namely increased absorption with increasing pH and a flattening of the curve above pH 6. In the case of proteoid roots however, there was a clear phosphate absorption peak between pH 4 and 5,5.

The above findings indicate that proteoid roots, particularly where phosphate absorption is concerned, fulfil an important function at the relatively low pH values of soils in which these plants normally occur.

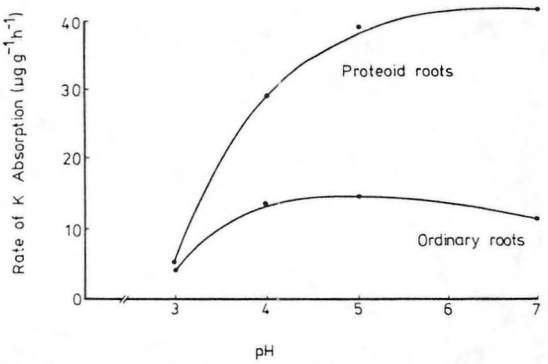

Figure 4 Rate of potassium absorption by roots of *Protea repens* (L.) L. at different pH values.

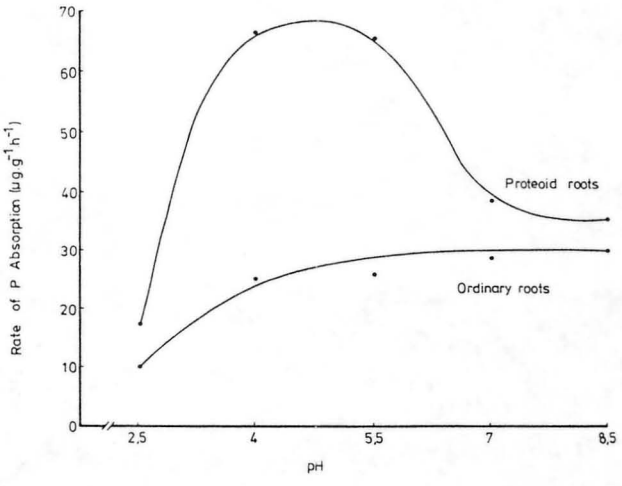

Figure 5 Rate of phosphate absorption by roots of *Protea compacta* R.Br. at different pH values.

Acknowledgements

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References

DU PREEZ, M., CARSTENS, J. & VAN WYK, E. 1981. Voorbereiding en droogverassing van blaarmonsters vir ontleding. N.I.V.V.-prosedure en tegnieke Nr. 32, N.I.V.V., Stellenbosch.

EPSTEIN, E. 1955. Passive permeation and active transport of ions in plant roots. *Pl. Physiol.* 30: 529 – 535.

EPSTEIN, E. 1961. The essential role of calcium in selective cation transport by plant cells. *Pl. Physiol.* 36: 437 – 444.

EPSTEIN, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Inc., New York..

EPSTEIN, E. & HAGEN, C.E. 1952. A kinetic study of the absorption of alkali cations by barley roots. *Pl. Physiol.* 27: 457 – 474.

EPSTEIN, E., RAINS, D.W. & ELZAM, O.E. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. *Proc. natn. Acad. Sci. U.S.A.* 49: 684 – 692.

GARDNER, W.K., BARBER, D.A. & PARBERY, D.G. 1982. Effect of microorganisms on the formation and activity of proteoid roots of *Lupinus albus* L. *Aust. J. Bot.* 30: 303 – 309.

GERSON, D.F. & POOLE, R.J. 1971. Anion absorption by plants: A unary interpretation of 'dual mechanisms'. *Pl. Physiol.* 48: 509 – 511.

GROVES, R.H. & KERAITIS, K. 1976. Survival and growth of seedlings of three sclerophyll species at high levels of phosphorus and nitrogen. *Aust. J. Bot.* 24: 681 – 690.

JACKMAN, R.H. 1965. The uptake of rubidium by the roots of some graminaceous and leguminous plants. *N.Z. Jl agric. Res.* 8: 763 – 777.

JEFFREY, D.W. 1967. Phosphate nutrition of Australian heath plants. 1. The importance of proteoid roots in *Banksia* (Proteaceae). *Aust. J. Bot.* 15: 403 – 411.

JONGENS-ROBERTS, S.M. 1981. Seasonal changes in biomass and phosphorus in *Leucospermum parile*. Fynbos biome project, fourth annual research meeting.

JOOSTE, J.H. 1973. Ysteropname deur wortel- en blaarweefsel van *Phaseolus vulgaris* L. Ph.D.-thesis, Univ. of Stellenbosch.

JOOSTE, J.H. & DE BRUYN, J.A. 1979. The dual mechanism of iron absorption in bean root and leaf tissues. *Jl S. Afr. Bot.* 45(3): 243 – 248.

LAMONT, B. 1972. The morphology and anatomy of proteoid roots in the genus *Hakea*. *Aust. J. Bot.* 20: 155 – 174.

LAMONT, B. 1980. Proteoid roots in South African Proteaceae. C.S.I.R. (C.S.P.) Fynbos biome project, second annual research meeting.

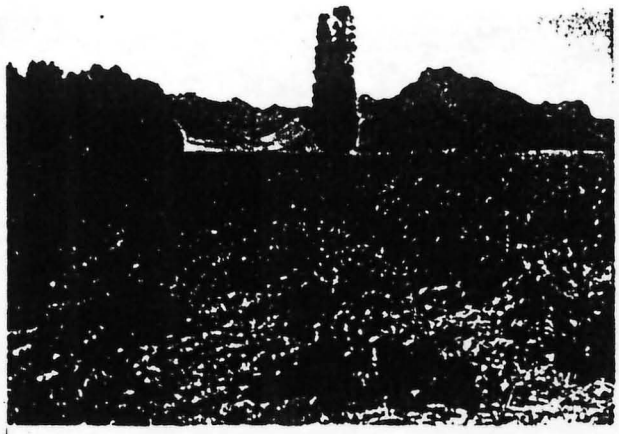
NISSEN, P. 1971. Uptake of sulfate by roots and leaf slices of barley: mediated by single, multiphase mechanisms. *Physiologia Pl.* 24: 315 – 324.

ORDIN, L. & JACOBSON, L. 1955. Inhibition of ion absorption and respiration in barley roots. *Pl. Physiol.* 30: 21 – 27.

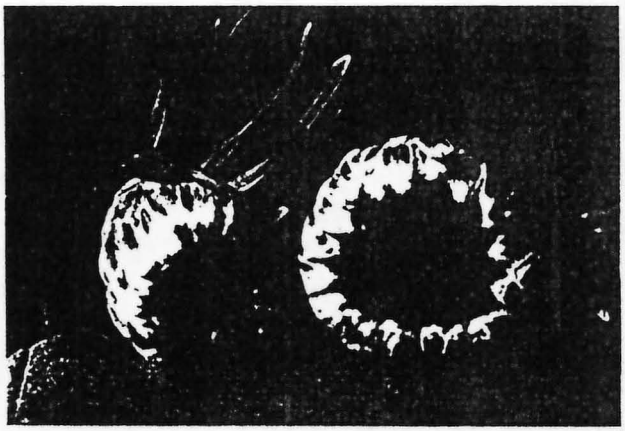
RAINS, D.W., SCHMID, W.E. & EPSTEIN, E. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. *Pl. Physiol.* 39: 274 – 278.

SUTCLIFFE, J.F. & BAKER, D.A. 1974. Plants and mineral salts. Edward Arnold Ltd, London.

WALTERS, C.M. 1980. Die verband tussen substraat en katioonkonsentrasie by die Proteaceae. M.Sc.-thesis, Univ. of Stellenbosch.



A clonal population of a hybrid between Leucospermum cordifolium *x* Leucospermum lineare *in full flower*

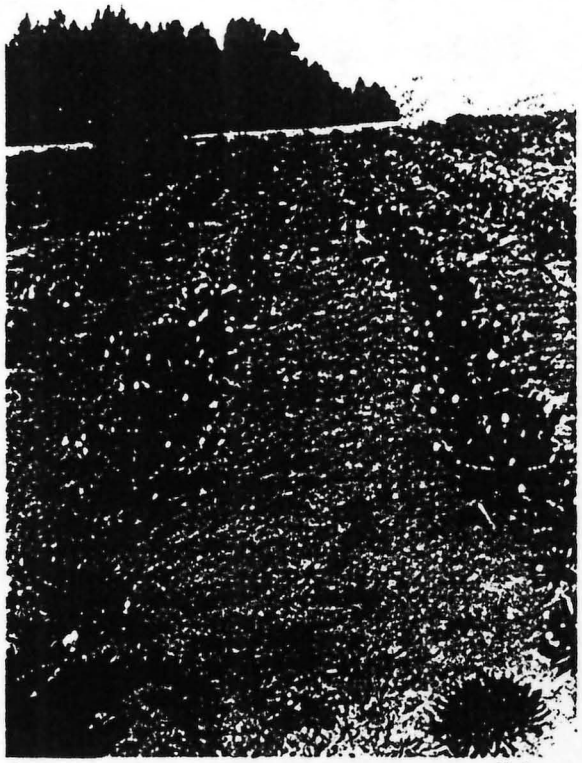


Examples of different selection of hybrids between Protea compacta *and* P. neriifolia *grown commercially at Protea Heights*

Protea Heights — cultivation for conservation

by Rob Soutter, Manager: SA Nature Foundation and G Jacobs, Department of Horticulture, University of Stellenbosch

THE CULTIVATION of proteas in plantations and gardens is an integral part of the conservation of the Cape's flora, and this branch of the wild flower industry promises to play an ever more vital conservationist role. A formal concern for the future of the Cape's flora was expressed through the establish-



A plantation of a hybrid between Leucospermum cordifolium *x* Leucospermum conocarpodendron. *Note the short stems typically of* Leucospermum conocarpodendron.

A plant of the same clone treated with a growth regulating chemical to stimulate shoot growth.

ment in 1920 of the Wild Flower Protection Society, with its call-to-arms for a "planting brigade not a plucking brigade". This had the aim of preventing the destruction and extinctions caused by indiscriminate picking of wild flowers — particularly Ericaceae and Proteaceae — for sale to the public.

Almost simultaneously, Kirstenbosch Botanic Garden had, since its inception seven years previously in 1913, managed to cultivate at least 10 protea species, and for the first time protea seeds were made available to members of the Botanical Society in 1922.

Cultivation was a logical answer to the need to reduce the impact of the flower pickers, but this concept has gained much wider support with the recent growth of a multi-million rand cutflower industry and consequent increased pressure on the veld.

The potential protective value of cultivation is illustrated by *Serruria florida*, once considered extinct and endemic to the Franschhoek area. Its rediscovery exposed the species to the depradations of flower pickers, but cultivation success achieved at Kirstenbosch ensured that the "blushing bride" protea became widely available, and nurseries now produce many times the volume found in the veld, where the species is now comparatively neglected and safe.

Admittedly cultivation is only part of the answer. Punitive measures and flower-picking permits also have key roles to play in flora conservation.

The advantages of intensive cultivation and plantations are that these measures are positive and that they offer financial gain — but only if it can be clearly demonstrated tht "planting" is cheaper than "plucking".

Intensive flower picking for short-term gain can be relatively cost-free in straight financial terms with no capital investment needed, but in ecological terms it is devastating. The temptation to pick and forego the long-term financial gains and reliability of plantation cultivation must be prevented.

This is where research plays an invaluable role, producing cultivars which flower exactly when the market requires them, offering the best colour and form to fit demand, standardised for easier packing, bred for longer lasting qualities and so well suited to their growing environment that propagation and disease problems are minimised. Thus they can compete with any other horticultural crop. The indifferent quality of blooms picked from the wild is then clearly displayed and cultivation becomes a distinctly better alternative.

Frank Batchelor

A far-sighted Stellenbosch farmer, Mr Frank Batchelor (1899-1977) was to understand these crucial points and to pioneer the development of South Africa's commercial protea industry. After growing a broad selection of deciduous fruits, he established, adjacent to his farm, a protea nursery which was to be his retirement hobby. This was on a hill later to be called Protea Heights.

By 1948 he was harvesting his first crop and the tremendous reception he received from an exhibit of his blooms at the Transvaal Horticultural Society's Spring Show a year later made him look more closely at the potential of the protea. He was soon to become the first person to produce cultivated proteas for the trade in South Africa, and then moved into rigorous selection, hybridisation and vegetative propagation of superior clones for local and overseas markets.

His achievements were recognised when in 1971 the South African Wild Flower Growers Association honoured this pioneer by establishing the Batchelor prize for the best hybrid protea produced for the cutflower industry. In 1976 Mr Batchelor willed Protea Heights to the S.A. Nature Foundation thus ensuring that pioneering work he had initiated would continue, supported by the research and facilities of modern scientific horticulture through Stellenbosch University's Department of Horticulture.

Scientifically managed wildflower cultivation, especially of Proteaceae, now produces about 15% of the blooms exported — a percentage which Protea Heights is committed to improving through research in laboratory and field. Improved hybrids will encourage more farmers to abandon "plucking" in favour of "planting", and veld-picked flowers will become uneconomical as they fail to match the rigorous quality demanded by the cutflower export market. Thus does cultivation become conservation.

In more formal economic terms, cultivation plays a valuable role within the economy, being labour intensive throughout the year offering unskilled and semi-skilled jobs, and in addition the wild-flower industry is worth several million rand in foreign exchange annually for South Africa.

The Nature Foundation considers Protea Heights and its work and results a superb example of sustainable utilisation — where conservation is not simply preservation but plays an active, positive role to demonstrate that conservation is for man and to ensure the safe future of our natural heritage.

Research

The proteas occurring naturally in the wild should be considered as the gene plasma resources, waiting to be developed to satisfy the ever increasing demand of a quality conscious overseas market.

It is generally said that a horticultural industry is only as good as its cultivars, and certainly in the case of floriculture one can add the rate at which new cultivars are placed on the market. For a sophisticated market such as the European flower market which is always wanting "something new" it is thus unlikely that the local protea industry will increase its share of the market if it is going to depend only on the limited number of species picked from the veld.

Breeding and selection of proteas is therefore a major programme at Protea Heights. This year alone some 300 hybrids of *Leucospermum* were screened for commercial usage. Some of the new and interesting hybrids raised include *L. glabrum* x (*L. cordifolium* x *L. lineare*), *L. pluridens* x (*L. cordifolium* x *L. lineare*) and *L. conocarpodendron* x *L. cordifolium*. A range of 14 *P. neriifolia* x *P. compacta* hybrids which flower from February to April was also screened during the last few years. Two of these selections were considered suitable for commercial production. The scope for developing new items is unlimited and it really depends on ourselves not to let this opportunity go by, as has happened with so many of our other indigenous flowers where the development was done overseas.

Although the development of new

cultivars is of prime importance, attention should also be given to other aspects of the commercial production of proteas. Vegetative propagation of new cultivars is an essential step for successful commercial protea production. A great deal of research on this subject has been done at Protea Heights. Except for some of the more "difficult-to-root" protea hybrids the technology of rooting cuttings on a commercial scale has been worked out and implemented at Protea Heights. Today all *Leucospermum* and *Protea* hybrids and selections are propagated by means of cuttings at Protea Heights.

Identifications of growth and developmental constraints of a cultivar which limit its production of economic biomass is the next logical step in a programme of cultivar development. Research at Protea Heights on this aspect will be illustrated by three examples:

(1) Plants of most *Leucospermum* cultivars become complex very rapidly. As from their third year plants produce excessive numbers of shoots which tend to be short and therefore not acceptable to the market. To overcome this problem, cultivars with this tendency are closely spaced (8 000 plants/ha) and then annually pruned so as to limit the number of shoots to about 30 stems per plant. This ensures a high production per hectare for the entire life of the plants.

(2) Hybrid cultivars which have *L. conocarpodendron* as one parent produce short stems irrespective of whether shoot number per plant is reduced. Although not yet in commercial usage great progress has been made in extending the shoot length of these cultivars with growth regulating chemicals.

(3) Extending the flowering time of *Leucospermum* will reduce the overproduction encountered in spring. Delaying the flowering time by disbudding of the primary inflorescences can delay the crop until December. Basic research on factors controlling flower initiation is in progress and it is anticipated that procedures will be developed to effectively control the time of flowering of this crop.

Successful post-harvest handling of a perishable commodity destined for a distant market is another important aspect which should be well developed if we want to secure our position on the overseas market. The post-harvest physiological abnormality of leaf blackening of many protea species and cultivars adds to the complexity of handling cut protea flowers. Blackening of leaves during transit of the flowers is induced primarily by poor temperature management of the flowers.

To minimize the likelihood of leaf blackening developing during transit it is important that the flowers be properly cooled before leaving the packhouse. For this reason Protea Heights has introduced a system of forced air precooling to safeguard against loss of quality of their product during transit. A practical solution to the development of leaf blackening in the vase must still materialize.

Controlling diseases and insects is particularly difficult when cultivation of a crop is undertaken in its natural habitat, and even more so when cultivation is based on clonally propagated cultivars. Protea Heights has played a significant role in assisting research workers in this field. Considerable progress has been made in the identification and control of diseases and pests. New genera and species of pathogenic fungi on proteas have been discovered.

The fact that Protea Heights is managed as a commercial cut-flower farm with its facilities and infrastructure available to research workers ensures that it will continue to play a significant role in the development of the cultivated protea.

RESEARCH INTO THE SOUTH AFRICAN PROTEACEAE

In the beginning . . .

by Marie Vogts

The year is 1940. Encouraged by beautiful protea blooms on plants I cultivated in the mountains and sorely discouraged by failures elsewhere, I am filled with an urge to find out why. No book on cultivation or propagation is of any help and I nervously approach the highest authority. "Young lady," Prof. Compton says, "you are wasting your time. The backlog on this kind of knowledge of the Proteaceae is too great. Forget about exploring reasons for their behaviour and their hidden characteristics and don't try to bring high mountain proteas down to open flats."

This was the challenge. Forty years ago there was no scientific guideline to explain the causes of the eccentric behaviour, reactions and needs of these plants. The only attempt to present classified knowledge was made by Joseph Knight in England almost two centuries ago. But as his work was limited to cultivation under highly artificial conditions, it could not serve as a basis for continuation of research and interest soon waned.

In South Africa only a few hints and odd bits of information could be gathered from botanic gardens and from the few individuals who had successfully cultivated single plants. Even these limited recommendations and inferences were based on reactions of proteas in the particular places where they had been cultivated, and these were all in the winter rainfall region. No one had been interested enough to spend time and money on investigating the causes of the obvious divergence from known horticultural crops. Nevertheless, I took up the challenge and launched a new approach to the problem, different from the age old standard trial and error method of accumulating preliminary knowledge through practice.

In the beginning of this scientific research the natural habitat served as a reference book. The apparent similarity of reactions to a number of factors of many species was so encouraging that the family was treated as a whole (in spite of a few glaring exceptions) and eventually 52 species, representing eight genera, were used for intensive investigation. I made numerous observations which seemed relative to the project, first in nature and then, by experiment, under control.

Since this research had to be considered as an undeveloped branch of science, analogy played a prominent role in the classification or grouping of what had been per-

16. Nighswander, J. E., and Patton, R. F. 1965. Epidemiology of the jack pine-oak gall rust (*Cronartium quercuum*) in Wisconsin. Can. J. Bot. 43:1561-1581.

17. Walkinshaw, C. H. 1978. Cell necrosis and fungus content in fusiform rust infected loblolly, longleaf, and slash pine seedlings. Phytopathology 68:1705-1710.

18. Walkinshaw, C. H., Dell, T. R., and Hubbard, S. D. 1980. Predicting field performance of slash pine families from inoculated greenhouse seedlings. U.S. For. Serv. Res. Pap. SO-160. 6 pp.

Phytophthora Root Rot of Commercially Cultivated Proteas in South Africa

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ABSTRACT

Von Broembsen, S. L., and Brits, G. J. 1985. Phytophthora root rot of commercially cultivated proteas in South Africa. Plant Disease 69:211-213.

Phytophthora cinnamomi was isolated from the roots of 63 species of diseased proteas (Proteaceae) in commercial fields in the South Western Cape Province of South Africa. Disease was often associated with poor soil drainage. Aboveground symptoms ranged from wilting and rapid death to chlorosis, decline, and eventual death. *P. cinnamomi* was associated most frequently with *Leucospermum* and *Leucadendron* spp. Pathogenicity of *P. cinnamomi* to indigenous proteas in eight genera (*Leucadendron, Leucospermum, Protea, Aulax, Brabeium, Mimetes, Paranomus,* and *Serruria*) was demonstrated by artificial inoculation.

Additional key words: disease control

Proteas (Proteaceae) produce striking flowers that remain attractive for weeks in the vase. Protea cultivation in South Africa has grown from a few hectares in 1960 to more than 2,000 ha today, making South Africa the world's leading producer and exporter of proteaceous cut flowers (B. Gibson, *personal communication*). About 20 indigenous species and several Australasian species are cultivated on a large scale. The most important production species are from two major indigenous genera, *Leucospermum* and *Protea*.

In 1976, we investigated extensive patch deaths of the common pincushion (*Leucospermum cordifolium* (Salisb. ex Knight) Fourc.) in a commercial cut flower planting on a site with poor drainage. Affected plants showed chlorosis, rapid wilting, and death. *Phytophthora cinnamomi* Rands was recovered from the dark brown, decayed roots of affected plants. Although *P. cinnamomi* has been reported previously from proteaceous hosts in both South Africa (6) and Australia (11), no studies on commercial plantings in these regions have been reported. *P. cinnamomi* has, however, recently been shown to cause root rot of *Banksia* spp. in Hawaii, where the disease is commercially important to cut flower production by this genus (2).

This paper reports the general occurrence of Phytophthora root rot on commercially cultivated proteas in the South Western Cape Province of South Africa and demonstrates the pathogenicity of *P. cinnamomi* to representative species of indigenous proteas.

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MATERIALS AND METHODS

Field study. Deaths of proteas grown in the South Western Cape Province for cut flower production, seed production, and breeding were investigated during 1976–1983. Disease patterns and symptom expression on different kinds of proteas were recorded. Tissues from the roots and collars of diseased plants were surface-disinfested in 0.1% NaOCl, rinsed in sterile distilled water, and plated on P10VP (5) or P10VPH (4) medium (both selective for pythiaceous fungi) and on Difco cornmeal agar. Isolations from soil samples taken from the root zones of diseased plants were made using a lupin baiting technique (1). Isolates were identified using the criteria of Waterhouse (9,10).

Pathogenicity tests. The pathogenicity of *P. cinnamomi* to 31 indigenous species of South African proteas (Table 1) was tested. Species selected from the major indigenous genera, *Leucospermum, Leucadendron*, and *Protea*, represented most of the subgenera within these genera and included most commercially important species. Single species from five minor genera were included in the tests to obtain an overall indication of the reaction of the indigenous component of the family Proteaceae to *P. cinnamomi*.

Inoculum was prepared by growing an A1 isolate (C11) of the mating type of *P. cinnamomi* from *Leucadendron argenteum* R. Br. in V-8 juice broth (3) for 15 days, then comminuting and diluting with sterile water to about 1,000 colony-forming units per liter. Previous inoculation studies had shown that a dosage of about 100 propagules per plant was likely to cause consistent infection of the most susceptible species but would also allow differences in susceptibility to be shown. One-year-old plants grown for

Table 1. Infection and death of indigenous South African Proteaceae after artificial inoculation with *Phytophthora cinnamomi*

Plant	Infected[a]	Dead[a]
Aulax cancellata	4	4
Brabeium stellatifolium	3	0
Leucadendron		
argenteum	4	4
nervosum	0	0
orientale	2	2
saligum	5	5
uliginosum	0	0
Leucospermum		
conocarpodendron	2	0
cordifolium	4	4
cuneiforme	4	2
formosum	0	0
glabrum	2	0
patersonii	5	5
praemorsum	5	5
prostratum	2	2
reflexum	2	0
Mimetes hirtus	1	0
Paranomus reflexus	1	0
Protea		
caffra	0	0
compacta	0	0
cynaroides	0	0
exima	0	0
lanceolata	1	0
laurifolia	0	0
magnifica	1	0
minor	2	0
neriifolia	0	0
nitida	0	0
obtusifolia	0	0
repens	0	0
Serruria florida	4	4

[a] Number of infected or dead plants of five inoculated.

10–12 mo in 2-L pots were inoculated by irrigating 100 ml of the mycelial suspension into the potting mixture (1:10, compost:sand). Controls were treated similarly, except sterile water was applied instead of mycelial inoculum. Treatments were replicated five times with single plants as experimental units.

Plants were held in a sand-filled shadehouse bed heated from beneath to 25 C and watered as necessary but otherwise exposed to the ambient conditions prevailing between December 1978 and May 1979 at Stellenbosch (midsummer to autumn). Symptom development and plant deaths were recorded weekly. Dead plants were removed when noted, their root systems examined, and isolations made as described before. After 5 mo, the experiment was terminated and isolations were made from the root systems of all surviving plants.

RESULTS

Field observations. Dark lesions occurred on the roots of all diseased plants and on the collars and lower stems of some. Cortical tissues were decayed but the stele remained intact. Proteoid roots (densely clustered rootlets characteristic of the Proteaceae) and secondary roots were often missing. For the most severely affected species, the disease was characterized by rapid wilting and death with subsequent retention of leaves on branches. Chlorosis sometimes preceded wilting. For less severely affected species, gradual decline accompanied by chlorosis and stunting preceded death. Although diseased plants occurred more frequently in poorly draining areas of a field, they also occcurred on scattered plants at sites with good drainage. Deaths occurred most frequently in the hot, dry period from summer to early autumn.

P. cinnamomi was isolated from 63 species of proteas in nine genera: *Aulax cancellata* (L.) Druce (=*pinifolia* Berg.); *Banksia burdetti* E. G. Bak., *B. coccinea* R. Br., *B. hookerana* Meisn.; *Brabeium stellatifolium* L.; *Leucadendron argenteum*, *L. comosum* (Thunb.) R. Br., *L. daphnoides* (Thunb.) Meisn., *L. discolor* Phill. & Hutch., *L. galpinii* Phill. & Hutch., *L. laureolum* (Lam.) Fourc., *L. meridianum* I. Williams, *L. microcephalum* (Gand.) Gand. & Schinz, *L. nobile* I. Williams, *L. orientale* I. Williams, *L. pubescens* R. Br., *L. rubrum* Burm. f., *L. salignum* Berg., *L. spissifolium* (Salisb. ex Knight) I. Williams, *L. tinctum* I. Williams, *L. tradouwense* I. Williams, *L. uliginosum* R. Br.; *Leucospermum attenuatum* (Burm. f.) Rourke, *L. catherinae* Compton, *L. conocarpodendron* (L.) Beuk, *L. cordifolium* 'Gold Dust,' *L. cordifolium* × *tottum* (L.) R. Br. 'Firefly,' *L. cordifolium* × *lineare* R. Br. 'Red Sunset,' *L. cuneiforme* (Burm. f.) Rourke, *L. erubescens* Rourke, *L. formosum* (Andr.) Sweet, *L. fulgens* Rourke, *L. glabrum* Phill., *L. grandiflorum* (Salisb.) R. Br., *L. lineare* R. Br., *L. muirii* Phill., *L. mundii* Meisn., *L. patersonii* Phill., *L. pluridens* Rourke, *L. praecox* Rourke, *L. praemorsum* (Meisn.) Phill., *L. prostratum* (Thunb.) Stapf, *L. reflexum* Buek ex Meisn., *L. tomentosum* (Thunb.) R. Br., *L. tottum* (L.) R. Br., *L. truncatum* (Buek ex Meisn.) Rourke, *L. utriculosum* Rourke, *L. vestitum* (Lam.) Rourke; *Mimetes cucullatus* (L.) R. Br., *Orothamnus zeyheri* Pappe ex Hook; *Paranomus reflexus* (Phill. & Hutch.) N. E. Br.; *Protea aurea* (Burm. f.) Rourke (=*longiflora* Lam.), *P. cynaroides* (L.) L., *P. effusa* E. Mey ex Meisn. (=*marlothii* Phill.), *P. grandiceps* Tratt., *P. lepidocarpodendron* (L.) L., *P. longifolia* Andr., *P. magnifica* Link, *P. nitida* Mill. (=*arborea* Houttuyn), *P. punctata* Meisn.; *Serruria florida* Knight; and *Telopea speciosissima* R. Br.

The most severe symptoms (rapid wilting and death with leaf retention) occurred on infected *Leucospermum*, *Leucadendron*, *Banksia*, *Serruria*, and *Aulax* spp. These symptoms were considered diagnostic for Phytophthora root rot on these species and were subsequently observed at many other locations where no isolations were attempted. Severe symptoms were found most frequently on *Leucospermum* and *Leucadendron* spp. Symptoms were generally less severe and developed more slowly on *Protea*, *Paranomus*, *Mimetes*, and *Telopea* spp.

Pathogenicity tests. *P. cinnamomi* isolate C11 was pathogenic to 19 of the 31 species that were artificially inoculated (Table 1). All plants from which the fungus was isolated had root symptoms similar to those of diseased plants in the field. Some of the infected plants wilted rapidly or became chlorotic and died. Other plants became infected but did not die during the 5-mo trial. Many *Leucospermum* and *Leucadendron* plants became infected and died suddenly. Few *Protea* plants became infected and none died.

DISCUSSION

P. cinnamomi is shown in this report for the first time to cause root rot of species in eight genera of South African proteas. Previously, *P. cinnamomi* was reported to be the cause of root and crown rot of silver trees (*Leucadendron argenteum*) (6); however, only aboveground stems were inoculated and no symptoms or reisolations from belowground parts were reported.

The field study showed important differences in disease severity and disease patterns among *Leucospermum*, *Leucadendron*, and *Protea* spp. *Leucospermum* and *Leucadendron* spp. were severely affected by Phytophthora root rot and appeared to be very susceptible. Although there was a correlation between poor drainage and disease incidence within a field for both of these species, disease also occurred at sites with good drainage. *Protea* spp. were less severely affected. Disease of mature *Protea* spp. rarely occurs at sites with good drainage. In fact, growers are using *Protea* spp. to replant sites where pincushions (*Leucospermum* spp.) have died from Phytophthora root rot.

The importance of Phytophthora root rot to the development of the South African cut flower industry is indicated by its occurrence on a wide range of commercially cultivated proteas and by its severity in the family Proteaceae as a whole. Plant loss was 52% in the 2-yr period after discovery of the disease in the original field of *Leucospermum cordifolium*. High plant losses usually force premature abandonment of the planting.

Disease control primarily consists of planting disease-free nursery material in sites with good drainage and no history of disease. Controlling the disease is particularly difficult in the South Western Cape Province because of abundant natural inoculum from infected indigenous flora and infested river water (7,8). Most new protea plantings are established on land cleared of native vegetation. Recovery of *P. cinnamomi* from soil at several of these newly cleared fields demonstrates the importance of this inoculum source (*unpublished*). Suitable methods for decontaminating river water are available but are costly for field use. Currently available fungicides tested in this region are not suitable for field control of Phytophthora root rot on *Leucospermum* spp. Control with fosetyl-Al (Aliette) is inadequate, and metalaxyl is unacceptably phytotoxic to *Leucospermum* spp. under field conditions (*unpublished*).

The variation in susceptibility observed among and within genera in this study suggests that some measure of control might be possible through use of resistant or tolerant varieties. Particularly within the genus *Protea*, breeding and selection for resistance appear promising. Within the genera *Leucospermum* and *Leucadendron*, resistant varieties are considerably less likely, but tolerance could be useful in a rootstock development program.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Chee, K. H., and Newhook, F. J. 1965. Improved methods for use in studies of *Phytophthora cinnamomi* Rands and other *Phytophthora* species. N.Z. J. Agric. Res. 8:88-95.
2. Cho, J. J. 1981. Phytophthora root rot of *Banksia*: Host range and chemical control. Plant Dis. 65:830-833.
3. Ribeiro, D. K. 1978. A Sourcebook on the Genus *Phytophthora*. J. Cramer, Lehr, Germany. 420 pp.
4. Tsao, P. H., and Guy, S. O. 1977. Inhibition of

Mortierella and *Pythium* in a *Phytophthora* isolation medium containing hymexazol. Phytopathology 67:796-801.
5. Tsao, P. H., and Ocana, G. 1967. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223:636-638.
6. Van Wyk, P. S. 1973. Root and crown rot of silver trees. J. S. Afr. Bot. 39:255-260.
7. Von Broembsen, S. L. 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South Western Cape Province. Phytophylactica 16:221-225
8. Von Broembsen, S. L. 1984. Distribution of *Phytophthora cinnamomi* in rivers of the South Western Cape Province. Phytophylactica 16:227-229.
9. Waterhouse, G. M. 1963. Key to the genus *Phytophthora* de Bary. Commonw. Mycol. Inst., Mycol. Pap. 92. 22 pp.
10. Waterhouse, G. M. 1970. The genus *Phytophthora* de Bary. Commonw. Mycol. Inst., Mycol. Pap. 122. 59 pp.
11. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monogr. 10. American Phytopathological Society, St. Paul, MN. 96 pp.

Factors Affecting Release of Ascospores by the Pear Scab Fungus (*Venturia pirina*)

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ABSTRACT

Latorre, B. A., Yáñez, P., and Rauld, E. 1985. Factors affecting release of ascospores by the pear scab fungus (*Venturia pirina*). Plant Disease 69:213-216.

Ascospores of *Venturia pirina* were monitored under field conditions in 1982 and 1983 with a Burkard 7-day recording spore trap adjusted to sample about 8 m^3 of air per hour at 55 cm above the ground. Ascospore productivity was determined weekly by sampling partially decomposed pear leaves near the spore trap. Ascospore emissions occurred mainly during daylight hours and fluctuated daily and seasonally (associated with periods of free moisture). The first mature ascospores were found when pear trees were in the green tip stage of fruit bud development (late August and early September). Maximum ascospore catches were recorded in September (white cluster to full bloom stage of fruit bud development), then progressively decreased until December. The lack of later liberation under Chilean conditions is apparently due to the absence of free moisture periods.

Pear scab, caused by *Venturia pirina* Aderh., is one of the most important diseases affecting pears (*Pyrus communis* L.) in Chile. It is particularly severe on Bartlett, Beurré du Bosc, Anjou, Packham's Triumph, and Winter Nelis. The fungus produces pseudothecia in fallen pear leaves. Mature ascospores are first released in late August or early September, when trees are at the green tip stage of fruit bud development (6). Thereafter, ascospores are normally released throughout the spring until late December. A similar ascospore discharge pattern has been reported for the apple scab fungus (*V. inaequalis* (Cke.) Wint.) in Chile (8). In some cultivars (eg, Winter Nelis), *V. pirina* may also survive as mycelium in infected twigs and produce conidia the following spring. Nevertheless, ascosporic inoculum appears to be a requisite for development of severe pear scab epidemics under Chilean conditions.

Factors affecting spore discharge have been well documented for the apple scab fungus. Ascospores of *V. inaequalis* are released only if pseudothecia are wetted (primarily by rains) and almost exclusively during daylight hours (2,4,5,7,9). Free moisture is also a major factor for ascospore release of *V. pirina* (1,10), but evidence for diurnal periodicity has not been conclusive. For instance in California, significant catches of ascospores were obtained during hours of darkness, suggesting that release of ascospores of *V. pirina* was not affected by light (1).

In this study, we report on seasonal fluctuation and diurnal periodicity of ascospore discharge of the pear scab fungus under field conditions.

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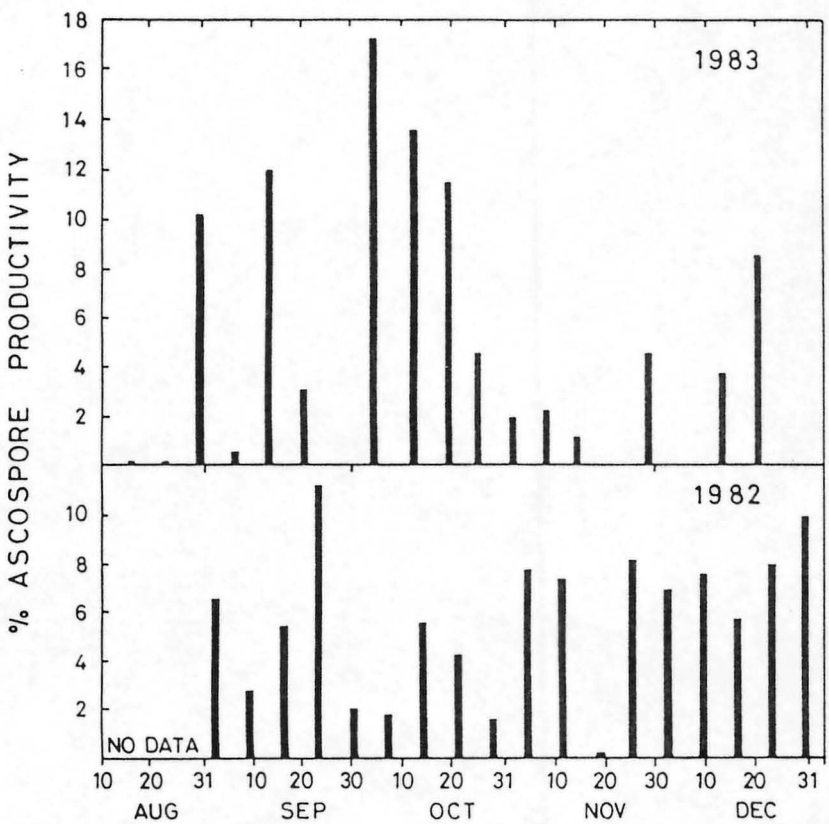

Fig. 1. Weekly ascospore productivity (percentage of seasonal total) of *Venturia pirina* in partially decomposed pear leaves, estimated by the procedure of Hirst and Stedman (5).

F01-1-FR-024

1.- AU: Rauch,-F-D; Nishimoto,-R-K; Parvin,-P-E
CA: Hawaii University Cooperative Extension Service.
Hawaii University Dept. of Horticulture.
TI: Protea [Leucospermum cordifolium, Protea eximia, Leucadendron discolor] weed control [Horticultural plants]
SO: Hortic-Dig-Dep-Hortic-Univ-Hawaii, May 1975, 22: 4.

2.- AU: Van-Wyk,-P-S
TI: Root and crown rot of silver trees. [Phytophthora cinnamomi, Leucadendron argenteum, Protea]
SO: J-S-Afr-Bot, July 1973, 39 (3): 255-260. Ref.

3.- AU: Lamont,-B.B.; Brown,-G.; Mitchell,-D.T.
TI: Structure, environmental effects on their formation, and function of proteoid roots in Leucadendron laureolum (Proteaceae).
SO: New-Phytol. London : Academic Press. July 1984. v. 97 (3) p. 381-390. ill.

4.- AU: DeFrank,-J.
TI: Weed control strategies for Hawaiian grown protea.
SO: Hortic-Dig-Univ-Hawaii-Coop-Ext-Serv. Honolulu, Hawaii : The Service. Oct 1988. (88) p. 5-9.

5.- AU: Berg,-G.C.-van-den.; Brits,-G.J.
TI: Development of Leucadendron single stem cut flowers.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 191-198.

6.- AU: Wright,-M.
TI: Integrated pest management--concepts and potential for the control of borers on proteas.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 153-157.

7.- AU: Jones,-R.; Faragher,-J.
TI: Cold storage of selected members of the Proteaceae and Australian native cut flowers.
SO: HortScience. Alexandria, Va. : American Society for Horticultural Science. Nov 1991. v. 26 (11) p. 1395-1397.

8.- AU: Silber,-A.; Ganmore-Neumann,-R.; Ben-Jaacov,-J.
TI: The response of Leucadendron 'Safari Sunset' to the fertilization regime.
SO: J-agric-sci. Cambridge : Cambridge University Press. Aug 2000. v. 135 (pt.1) p. 27-34.

9.- AU: Silber,-A.; Ganmore-Neumann,-R.; Ben-Jaacov,-J.
TI: The response of three Leucadendron cultivars (Proteaceae) to phosphorus levels.
SO: Sci-hortic. Amsterdam : Elsevier Science B.V. Apr 28, 2000. v. 84 (1/2) p. 141-149.

10.- AU: Canmore-Neumann,-R.; Silber,-A.; Mitchnick,-B.; Gilad,-S.; Ben-Jaacov,-J.
TI: Effect of pH on the development of Leucadendron 'Safari Sunset'.
SO: Acta-hortic. Leuven, Belgium : International Society for Horticultural Science. Dec 1997. (453) p. 47-52.

11.- TI: Field studies on the effectiveness of phosphonate suppression of Phytophthora root rot in proteas.
AU: Turnbull-LV; Crees-LR; Brits-GJ (ed.); Wright-MG
AD: Redlands Research Station, Queensland Department of Primary Industries, Queensland, Australia.
SO: Third international Protea research symposium, Harare, Zimbabwe, 10-15 Oct. 1993. Acta-Horticulturae. 1995, No. 387, 141-151; 16 ref.

12.- TI: Development of Leucadendron single stem cut flowers.
AU: Berg-GC-van-den; Brits-GJ; Van-den-Berg-GC; Brits-GJ (ed.); Wright-MG
AD: Fynbos Research Unit, Vegetable and Ornamental Plant Institute, Private Bag X1, 7607 Elsenburg, South Africa.
SO: Third international protea research symposium, Harare, Zimbabwe, 10-15 October 1993. Acta-Horticulturae. 1995, No. 387, 191-198; 3 ref.

13.- TI: Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of Protea.
AU: Malan-DG; Roux-RD-le; Le-Roux-RD; Brits-GJ (ed.); Wright-MG
AD: VOPI Fynbos Unit, Agricultural Research Council, Private Bag X1, Elsenburg, 7607, South Africa.
SO: Third international protea research symposium, Harare, Zimbabwe, 10-15 October 1993. Acta-Horticulturae. 1995, No. 387, 91-97; 1 ref.

14.- TI: Irrigation requirements of proteas in the moderate climatic zones of Tenerife.
AU: Saenz-Pisaca-D; Bravo-Gonzalez-S
AD: Universidad de La Laguna, Tenerife, Canary Islands.
SO: Ciclo de seminarios: VII Curso Internacional de Riego Localizado. 1996, 103-111; 8 ref.

15.- TI: Leaf chemical composition and nutrient removal by stems of Leucadendron cvv. Silvan Red and Safari Sunset.
AU: Cecil-JS; Barth-GE; Maier-NA; Chvyl-WL; Bartetzko-MN
AD: South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia.
SO: Australian-Journal-of-Experimental-Agriculture. 1995, 35: 4, 547-555; 26 ref.

16.- TI: Sucrose prevents foliage desiccation in cut Leucadendron `Silvan Red' during cool storage.
AU: Jones-RB
AD: Institute for Horticultural Development, Knoxfield, Victorian Department of Agriculture, Private Bag 15, South Eastern Mail Centre, Vic. 3176, Australia.
SO: Postharvest-Biology-and-Technology. 1995, 6: 3-4, 293-301; 21 ref.

17.- TI: Growing proteas commercially.
AU: Gollnow-B
AD: NSW Agriculture, Locked Bag 11, Windsor, NSW 2756, Australia.
SO: Agfacts -Department-of-Agriculture,-New-South-Wales. 1995, No. H9.1.19, 11 pp.; 13 col. pl.; 11 ref.

18.- TI: Predicting vase life in tropical cut flowers and foliage.
AU: Hansen-JD; Paull-RE; Hara-AH; Tenbrink-VL
AD: ARS-USDA, 13601 Old Cutler Road, Miami, FL 33158, USA.
SO: Proceedings of the 104th annual meeting of the Florida State Horticultural Society, Miami Beach, Florida, 29-31 Oct. 1991. Proceedings-of-the-Florida-State-Horticultural-Society. 1991, publ. 1992, 104: 61-63; 10 ref.

19.- TI: A pre-storage sucrose pulse protects cut Leucadendron var. 'Silvan Red' during long term dry storage at 1°C.
AU: Jones-RB
AD: Institute of Plant Sciences, Knoxfield, PO Box 174, Ferntree Gully, Vic. 3156, Australia.
SO: Acta-Horticulturae. 1991, No. 298, 247-253; Hortifroid. Fifth international symposium on postharvest physiology of ornamental plants. Importance of cold in ornamental horticulture, Nice, France, 11-15 Mar. 1991.; 6 ref.

20.- TI: Evaluation of pre-emergence herbicides on four proteaceous species.
AU: DeFrank-J; Easton-Smith-VA
AD: Department of Horticulture, 3190 Maile Way, Honolulu, HI 96822, USA.
SO: Tropical-Agriculture. 1990, 67: 4, 360-362; 4 ref.

21.- TI: Introduction of proteas for cut flower and foliage in Tenerife.
AU: Rodriguez-Perez-JA
AD: Jardin de Aclimatacion de la Orotava, Centro de Investigacion y Tecnologia Agraria, 38400 Puerto de la Cruz, Tenerife, Canary Islands.
SO: Acta-Horticulturae. 1989, No. 246, 265-267; 1 map, International symposium on protected cultivation of ornamentals in mild winter climates, Tenerife, Canary Islands, 18-21 Oct., 1988; 4 ref.

22.- TI: New approaches to the development of proteaceous plants as floricultural commodities.
AU: Ben-Jaacov-J; Ackerman-A; Gilad-S; Shchori-Y
AD: Department of Floriculture, ARO, Volcani Center, Bet Dagan 50-250, Israel.
SO: Acta-Horticulturae. 1989, No. 252, 193-199; Symposium on the development of new floricultural crops, Faaborg, Denmark, 28 Aug.-2 Sep., 1988; 16 ref.
DE: Growing-media; cut-flowers; production-; soilless-culture; Grafting-; cultural-methods; ornamental-plants; ornamental-woody-plants

23.- TI: Control of experimental Phytophthora cinnamomi stem infections of Rhododendron, Leucadendron and Eucalyptus by dimethomorph, fosetyl-Al and metalaxyl.
AU: Marks-GC; Smith-IW
AD: Department of Conservation, Forests and Lands, 378 Cotham Road, Kew, Vic. 3101, Australia.
SO: Australian-Journal-of-Experimental-Agriculture. 1990, 30: 1, 139-143; 6 ref.

24.- TI: Post-harvest water relations of Leucadendron cv. Silvan Red.
AU: Street-KA; Sedgley-RH
AD: Crop and Pasture Science Group, School of Agriculture, University of Western Australia, Nedlands, WA 6009, Australia.
SO: Acta-Horticulturae. 1990, No. 264, 109-113; Second International Protea Research Symposium, San Diego, California, USA, 7-8 March, 1989.; 6 ref.

25.- TI: Improved methods for rooting cuttings of Protea obtusifolia.
AU: Faruchi-Y; Ackerman-A; Gilad-S; Ben-Jaacov-J; Riov-J; Littlejohn-GM (ed.); Hettasch-H
AD: Department of Ornamental Horticulture, ARO, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.
SO: Proceedings of the fourth international Protea working group symposium, Jerusalem, Israel, 17-21 March 1996. Acta-Horticulturae. 1997, No. 453, 153-157; 5 ref.

26.- TI: Fungi occurring on Proteaceae. I.
AU: Swart-L; Crous-PW; Denman-S; Palm-ME
AD: ARC, Private Bag X1, Elsenburg, 7606 South Africa.
SO: South-African-Journal-of-Botany. 1998, 64: 2, 137-145; 30 ref.

27.- TI: Yield and seasonal growth flushing of Protea 'Pink Ice' and Leucadendron 'Silvan Red' in South Australia.
AU: Barth-GE; Maier-NA; Cecil-JS; Chyvl-WL; Bartetzko-MN
AD: South Australian Research and Development Institute, Plant Research Centre, Waite Research Precinct, GPO Box 397, Adelaide, SA 5001, Australia.
SO: Australian-Journal-of-Experimental-Agriculture. 1996, 36: 7, 869-875; 13 ref.

28.- TI: Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of Protea.
AU: Malan-DG; Roux-RD-le; Le-Roux-RD; Brits-GJ (ed.); Wright-MG
AD: VOPI Fynbos Unit, Agricultural Research Council, Private Bag X1, Elsenburg, 7607, South Africa.
SO: Third international protea research symposium, Harare, Zimbabwe, 10-15 October 1993. Acta-Horticulturae. 1995, No. 387, 91-97; 1 ref.

29.- TI: Pruning of Protea cv. Cardinal to optimise economic biomass production.
AU: Gerber-AI; Greenfield-EJ; Theron-KI; Jacobs-G; Brits-GJ (ed.); Wright-MG
AD: Department of Horticultural Science, University of Stellenbosch, Stellenbosch 7600, South Africa.
SO: Third international protea research symposium, Harare, Zimbabwe, 10-15 October 1993. Acta-Horticulturae. 1995, No. 387, 99-106; 8 ref.

30.- TI: Gevuina nut (Gevuina avellana, Proteaceae), a cool climate alternative to macadamia.
AU: Halloy-S; Grau-A; McKenzie-B
AD: Crop and Food Research Institute, Invermay Agricultural Research Centre, Private Bag 50034, Mosgiel, New Zealand.

SO: Economic-Botany. 1996, 50: 2, 224-235; 2 pl., 2 maps; 51 ref.

31.- TI: Effect of sampling time and leaf position on leaf nutrient composition of Protea 'Pink Ice'.
AU: Maier-NA; Barth-GE; Cecil-JS; Chvyl-WL; Bartetzko-MN
AD: South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia.
SO: Australian-Journal-of-Experimental-Agriculture. 1995, 35: 2, 275-283; 28 ref.

32.- TI: Protea diseases and their control.
AU: Forsberg-L
SO: 1993, 16 pp.

33.- TI: Effect of pruning on growth and flowering response of Protea cv. Carnival.
AU: Greenfield-EJ; Theron-KI; Jacobs-G
AD: Department of Horticultural Science, University of Stellenbosch, Private Bag X5018, 7599 Stellenbosch, South Africa.
SO: Journal-of-the-Southern-African-Society-for-Horticultural-Sciences. 1994, 4: 1, 42-46; 5 ref.
DE: pruning-; seasons-; flowers-; development-; cut-flowers; production-; cultural-methods; ornamental-plants; ornamental-woody-plants

34.- TI: Cold storage of selected members of the Proteaceae and Australian native cut flowers.
AU: Jones-R; Faragher-J
AD: Institute of Plant Sciences, Knoxfield, PO Box 174, Ferntree Gully, Vic. 3156, Australia.
SO: HortScience. 1991, 26: 11, 1395-1397; 14 ref.

35.- TI: Ant control on protea in Hawaii.
AU: Hara-AH; Hata-TY
AD: Department of Entomology, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 461 West Lanikaula Street, Hilo, HI 96720, USA.
SO: Scientia-Horticulturae. 1992, 51: 1-2, 155-163; 7 ref.

36.- AU: Berg,-G.C.-van-den.; Brits,-G.J.
TI: Development of Leucadendron single stem cut flowers.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 191-198.

37.- AU: Wright,-M.
TI: Integrated pest management--concepts and potential for the control of borers on proteas.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 153-157.

38.- AU: Turnbull,-L.V.; Crees,-L.R.
TI: Field studies on the effectiveness of phosphonate suppression of Phytophthora root rot in proteas.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 141-151.

39.- AU: Gerber,-A.I.; Greenfield,-E.J.; Theron,-K.I.; Jacobs,-G.
TI: Pruning of Protea cv. Carnival to optimise economic biomass production.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 99-106.

40.- AU: Malan,-D.G.; Le-Roux,-R.E.
TI: Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of Protea.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 91-97.

41.- AU: Montarone,-M.; Allemand,-P.
TI: Growing Proteaceae soilless under shelter.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 73-84.

42.- AU: Allemand,-P.; Montarone,-M.; Le-Bris,-M.
TI: Architectural structure of two species of Protea grown in soilless cultivation.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) p. 63-71.

43.- AU: Brits,-G.J.-ed.; Wright,-M.G.-ed.
TI: Third International Protea Research Symposium.
SO: Acta-hortic. Wageningen : International Society for Horticultural Science. June 1995. (387) 207 p.

44.- AU: Maier,-N.A.; Barth,-G.E.; Cecil,-J.S.; Chvyl,-W.L.; Bartetzko,-M.N.
TI: Effect of sampling time and leaf position on leaf nutrient composition of Protea 'Pink Ice'.
SO: Aust-j-exp-agric. East Melbourne, Vic. Australia : Commonwealth Scientific and Industrial Research Organization, c1985-. 1995. v. 35 (2) p. 275-283.

45.- AU: Forsberg,-Leif.
CA: Queensland. Dept. of Primary Industries.
TI: Protea diseases and their control.
SO: Brisbane : Queensland Govt., Dept. of Primary Industries, c1993. 13 p. : col. ill.

46.- AU: Matthews,-Lewis-J.
TI: The protea growers handbook for New Zealanders.
SO: Auckland, N.Z. : David Bateman, 1993. 128 p., [8] p. of plates : ill. (some col.)

47.- AU: Reid,-M.S.; Van-Doorn,-W.; Newman,-J.P.
TI: Leaf blackening in Proteas.
SO: Acta-Hortic. Wageningen : International Society for Horticultural Science. Dec 1989. (261) p. 81-84.

48.- AU: Paull,-R.E.; Dai,-J.W.
TI: Protea postharvest black leaf a problem in search of a solution.
SO: Acta-Hortic. Wageningen : International Society for Horticultural Science. Apr 1990. (264) p. 93-101.

49.- AU: Dupee,-S.A.; Goodwin,-P.B.

TI: Effect of temperature, daylength and growth regulators on flowering in Protea, Telopea and Leucospermum.
SO: Acta-Hortic. Wageningen : International Society for Horticultural Science. Apr 1990. (264) p. 79-86.

50.- AU: Dupee,-S.A.; Goodwin,-P.B.
TI: Flower initiation in Protea and Telopea.
SO: Acta-Hortic. Wageningen : International Society for Horticultural Science. Apr 1990. (264) p. 71-77.

51.- AU: Rodriguez-Perez,-J.A.
TI: A technique to improve the propagation by stem cuttings of Protea obtusifolia Buek ex Meisn.
SO: Acta-Hortic. Wageningen : International Society for Horticultural Science. Apr 1990. (264) p. 41-44.

52.- AU: DeFrank,-J.
TI: The response of nine protea species to spray applications of fluazifop-p.
SO: Trop-Pest-Manage. London : Taylor & Francis. Apr/June 1990. v. 36 (2) p. 145-146.

53.- AU: MacFarlane,-J.R.; Franz,-P.R.
TI: Post harvest disinfestation of export proteas.
SO: Plant-Prot-Q. Victoria : R.G. Richardson. Quarterly 1989. v. 4 (2) p. 73-74.

54.- AU: Gouws,-L.; Jacobs,-G.; Strydom,-D.K.
TI: Factors affecting rooting and auxin absorption in stem cuttings of protea.
SO: J-Hortic-Sci. Ashford : Headley Brothers Ltd. Jan 1990. v. 65 (1) p. 59-63.

55.- AU: Grose,-M.J.
TI: Phosphorus nutrition of seedlings of the waratah, Telopea speciosissima (Sm.) R.Br. (Proteaceae).
SO: Aust-J-Bot. East Melbourne : Commonwealth Scientific and Industrial Research Organization. 1989. v. 37 (4) p. 313-320.

56.- AU: Witkowski,-E.T.F.
TI: Effects of nutrients on the distribution of dry mass, nitrogen and phosphorus in seedlings of Protea repens (L.) L. (Proteaceae).
SO: New-Phytol. New York, N.Y. : Cambridge University Press. Aug 1989. v. 112 (4) p. 481-487.

57.- AU: DeFrank,-J.
TI: Weed control strategies for Hawaiian grown protea.
SO: Hortic-Dig-Univ-Hawaii-Coop-Ext-Serv. Honolulu, Hawaii : The Service. Oct 1988. (88) p. 5-9.

58.- AU: Paull,-R.E.
TI: Protea postharvest black leaf.
SO: Hortic-Dig-Univ-Hawaii-Coop-Ext-Serv. Honolulu, Hawaii : The Service. Oct 1988. (88) p. 3-4.

59.- AU: Paulin,-G.
TI: Protea growing in New Zealand.
SO: Res-Ext-Ser-Coll-Trop-Agric-Hum-Resour-Univ-Hawaii-Coop-Ext-Serv. Honolulu, Hawaii : The Service. Feb 1982. (016) p. 13-16.

60.- AU: Smith,-A.J.; Jooste,-J.H.
TI: Phosphate absorption by excised ordinary and proteoid roots of Protea compacta R. Br.
SO: S-Afr-J-Bot-Off-J-S-Afr-Assoc-Botan-S-Afr-Tydskr-Plantkd-Amptelike-Tydskr-S-Afr-Genoot-Plantkd. Pretoria, S. Africa : Bureau for Scientific Publications. Dec 1986. v. 52 (6) p. 549-551.

61.- AU: Brits,-G.J.
TI: Germination depth vs. temperature requirements in naturally dispersed seeds of Leucospermum cordifolium and L. cuneiforme (Proteaceae).
SO: S-Afr-J-Bot-Off-J-S-Afr-Assoc-Botan-S-Afr-Tydskr-Plantkd-Amptelike-Tydskr-S-Afr-Genoot-Plantkd. Pretoria, S. Africa : Bureau for Scientific Publications. Apr 1987. v. 53 (2) p. 119-125. ill.

62.- AU: Matthews,-L.J.
TI: Growing proteas in New Zealand.
SO: Pac-Hortic. San Francisco : Pacific Horticultural Foundation. Spring 1986. v. 47 (1) p. 38-41. ill.

63.- AU: Parvin,-P.E.
TI: Protea production in Hawaii.
SO: Pac-Hortic. San Francisco : Pacific Horticultural Foundation. Winter 1985. v. 46 (4) p. 18-21. ill.

64.- AU: Vorster,-P.W.; Jooste,-J.H.
TI: Translocation of potassium and phosphate from ordinary and proteoid roots to shoots in the Proteaceae.
SO: S-Afr-J-Bot-S-Afr-Tydskr-Plantkd. Pretoria, S. Africa : Bureau for Scientific Publications. Aug 1986. v. 52 (4) p. 282-285. ill.

65.- AU: Vorster,-P.W.; Jooste,-J.H.
TI: Potassium and phosphate absorption by excised ordinary and proteoid roots of the Proteaceae.
SO: S-Afr-J-Bot-S-Afr-Tydskr-Plantkd. Pretoria, S. Africa : Bureau for Scientific Publications. Aug 1986. v. 52 (4) p. 277-281.

66.- AU: Faragher,-J.D.
TI: Effects of cold storage methods on vase life and physiology of cut waratah inflorescences (Telopea speciosissima, Proteaceae).
SO: Sci-Hortic. Amsterdam : Elsevier Science Publishers. June 1986. v. 29 (1/2) p. 163-171.

67.- AU: Faragher,-J.D.
TI: Post-harvest physiology of waratah inflorescences (Telopea speciosissima, Proteaceae).
SO: Sci-Hortic. Amsterdam : Elsevier Science Publishers. Apr 1986. v. 28 (3) p. 271-279. ill.

68.- AU: Collett,-R.
TI: Proteas: our fifteen years of experience.

SO: Pac-Hortic. San Francisco : Pacific Horticultural Foundation. Fall 1985. v. 46 (3) p. 32-38. ill.

69.- AU: Soutter,-R.; Jacobs,-G.
TI: Protea Heights--cultivation for conservation.
SO: Veld-Flora. Kirstenbosch : Botanical Society of South Africa. Dec 1984. v. 70 (4) p. 99-101. ill.

70.- AU: Asper,-H.
TI: Propagation of proteas.
SO: Comb-Proc-Int-Plant-Propagators-Soc. Boulder : The Society. 1984 (pub. 1985). v. 34 p. 168-169.

71.- AU: Von-Broembsen,-S.L.; Brits,-G.J.
TI: Phytophthora root rot of commercially cultivated proteas in South Africa.
SO: Plant-Dis. St. Paul, Minn. : American Phytopathological Society. Mar 1985. v. 69 (3) p. 211-213.

72.- AU: Lamont,-B.B.; Brown,-G.; Mitchell,-D.T.
TI: Structure, environmental effects on their formation, and function of proteoid roots in Leucadendron laureolum (Proteaceae).
SO: New-Phytol. London : Academic Press. July 1984. v. 97 (3) p. 381-390. ill.

73.- AU: Lamont,-B.
TI: Proteoid roots in the South African Proteaceae Plant morphology.
SO: J-S-Afr-Bot. Kirstenbosch : National Botanic Gardens of South Africa. Apr 1983. v. 49 (pt.2) p. 103-123. ill.

74.- AU: Yoshimoto,-S.
TI: A progress report on protea research Leucospermum cordifolium, propagation, flower bud development, pot plants, Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 1-5.

75.- AU: Mau,-R.F.L.
TI: Insect and mite pests of protea Includes guidelines for control in Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 6-10.

76.- AU: Cho,-J.J.
TI: Diseases of protea: root rot caused by Meloidogyne incognita and Phytophthora Protea spp., Leucospermum cordifolium, Banksia spp., Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 11-17. ill.

77.- AU: Parvin,-P.E.
TI: Propagation Protea spp., Banksia spp., Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 18-19.

78.- AU: Parvin,-P.E.; Leonhardt,-K.W.
TI: Care and handling of cut protea flowers Leucospermum, Protea, Banksia, Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 20-23.

79.- AU: Smith,-M.R.; Yang,-K.P.; Liang,-T.
TI: Protea post-harvest handling and processing Cut flowers, transportation, packaging, Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 38-39.

80.- AU: Cho,-J.J.
TI: Biology and control of Protea diseases Protea, Leucospermum, Banksia, Hawaii.
SO: Res-Ext-Ser-Hawaii-Inst-Trop-Agric-Hum-Resour. Honolulu : The Institute. July 1982. (018) p. 40-44.

81.- AU: Greenhalgh,-F.C.
TI: Evaluation of fungicides for control of root rot of proteaceous plants, 1979 Protea (Protea repens), leucadendron (Leucadendron discolor), root rot; Phytophthora cinnamomi.
SO: Fungic-Nematic-Tests-Results-Am-Phytopathol-Soc. s.l., The Society. 1980. v. 35 p. 142.

82.- AU: Paull,-R.; Criley,-R.A.; Goo,-T.; Parvin,-P.E.
TI: Leaf blackening in cut Protea eximia: importance of water relations.
SO: Second International Symposium on Post Harvest Physiology of Cut Flowers, Davis, California, USA, 21-25 July 1980 / convenors, A.M. Kofranek, M.S. Reid. Second International Symposium on Post Harvest Physiology of Cut Flowers, Davis, California, USA, 21-25 July 1980 / convenors, A.M. Kofranek, M.S. Reid. The Hague : International Society for Horticulture Science, 1981. The Hague : International Society for Horticulture Science, 1981. p. 159-166. ill.

83.- TI: Protea Workshop; proceedings, 7th annual, Maui Community College, March 15, 1979.
SO: Misc-Publ-Hawaii-Univ-Coop-Ext-Serv. Honolulu, The Service. Jan 1980. (176) 28 p. ill.

84.- AU: Parvin,-P.E
CA: Protea Workshop, 7th, Maui Community College, 1979.
TI: The art and science of growing proteas--current recommendations.
SO: Misc-Publ-Hawaii-Univ-Coop-Ext-Serv. Honolulu, The Service. Jan 1980. (176) p. 16-20.

85.- AU: Akamine,-E.K.; Goo,-T.; Suehisa,-R.
TI: Relationship between leaf darkening and chemical composition of leaves of species of cut flowers of Protea.
SO: Florists-Rev. Chicago Feb 8, 1979. v. 163 (4236) p. 62-63, 107-108.

86.- AU: Lamont,-B
TI: Proteoid roots: root systems in the family Proteaceae and their relevance to horticulture
SO: Aust-Plants, Sept 1977, 9 (72): 161-164.

87.- AU: Lewis,-O-A-M; Stock,-W-D
TI: A preliminary study of the nitrogen nutritional status of members of the South African Proteaceae [Leucadendron, Protea, Brabeium]
SO: J-S-Afr-Bot, Apr 1978, 44 (2): 143-151.

88.- AU: Cho,-J-J
CA: Hawaii University Cooperative Extension Service.
TI: Progress report on Protea disease research [Meloidogyne incognita, Botrytis cinerea, Phytophthora cinnamoni, Rhizoctonia solani]
SO: Misc-Publ-Hawaii-Univ-Coop-Ext-Serv, May 1978, 157: 7-11.
89.- AU: Rauch,-F-D; Nishimoto,-R-K; Parvin,-P-E
CA: Hawaii University Cooperative Extension Service.
Hawaii University Dept. of Horticulture.
TI: Protea [Leucospermum cordifolium, Protea eximia, Leucadendron discolor] weed control [Horticultural plants]
SO: Hortic-Dig-Dep-Hortic-Univ-Hawaii, May 1975, 22: 4.
90.- AU: Teague,-W
TI: Growing proteas [Proteaceae] in southern California
SO: Comb-Proc-Int-Plant-Propag-Soc, 1974, 24: 43-44.
91.- AU: McKenzie,-B-L
TI: Propagation of Proteaceae by cuttings
SO: Comb-Proc-Int-Plant-Propag-Soc, 1973, 23: 380.
92.- AU: Hanekom,-A-N; Deist,-J; Blommaert,-K-L-J
TI: Seasonal uptake of phosphorus-32 [radioactive phosphorus] an d rubidium-86 [radioactive rubidium] by Protea cynaroides (L.) L
SO: Agroplantae, 1973, 5 (4): 107-110. Ref. Eng. sum.
93.- AU: Kroon-GH
TI: Evaluation of Leucocoryne as a new cut flower
SO: Prophyta. 1989, 43: 1, 15-16; 3 ref.
AN: 901610343
94.- AU: Frolich, E.F. 1966
TI: Rooting citrus and avocado cuttings
SO: Pro. Inter. Plant. SOC: 16: 51-54
95.- AU: Frolich, E.F. 1972
TI: Use of the etiolation technique in rooting avocado cuttings
SO: Calif. Avoc. Soc. Yearbook, 1971-1972: 91-109
96.- AU: Claassens, A. S. 1981
TI: Soil preparation and fertilisation of proteas
SO: Flowers and Ornamental Shrubs B. 14/1981. Departament of Agric. And Fisheriss, Pretoria, South Africa
97.- AU: Conijn,-C.G.M.
TI: New fungicide for the control of Stagonospora in Amaryllis [Hippeastrum, mold, Stagonospora curtisii, Netherlands]. Nieuw stagonospora-bestrijdingsmiddel in Amaryllis.
SO: Vakbl-Bloemisterij. Den Haag, Netherlands : C. Misset. Apr 22, 1983. v. 38 (16) p. 29. ill.
98.- AU: Turian,-G.
TI: Decreasing pH-gradient toward the apex of germinating pollen tubes Daffodil, Narcissus pseudonarcissus, amaryllis, Hippeastrum vittatum.
SO: Ber-Schweiz-Bot-Ges-Bull-Soc-Bot-Suisse. Teufen, Switzerland, KRYPTO. 1981. v. 91 p. 161-167. ill.
99.- AU: Arroyo,-S.
TI: The chromosomes of Hippeastrum, Amaryllis and Phycella (Amaryllidaceae) Amaryllis belladonna.
SO: Kew-Bull. London, Her Majesty's Stationery Office. 1982. v. 37 (2) p. 211-216. ill.
100.- AU: Seabrook,-J-E-A; Cumming,-B-G
TI: The in vitro propagation of Amaryllis (Hippeastrum spp. hybrids) [Tissue culture]
SO: In Vitro J Tissue Cult Assoc, Dec 1977, 13 (12): 831-836. Ref.
101.- AU: Hong,-Y-P
TI: The effect of temperature treatment for flowering and growth on Amaryllis (Hippeastrum hybridum)
SO: Korea-Repub-Nongch'on-Jin-Heung-Chung-Res-Rep-Hortic, Dec 1970, 13: 57-63.
102.- AU: Kaku,-H.; Van-Damme,-E.J.M.; Peumans,-W.J.; Goldstein,-I.J.
TI: Carbohydrate-binding specificity of the daffodil (Narcissus pseudonarcissus) and amaryllis (Hippeastrum hybr.) bulb lectins.
SO: Arch-Biochem-Biophys. Duluth, Minn. : Academic Press. June 1990. v. 279 (2) p. 298-304.
103.- AU: Mogensen,-H.L.
TI: Juxtaposition of the generative cell and vegetative nucleus in the mature pollen grain of amaryllis (Hippeastrum vitatum).
SO: Protoplasma. Wien, Austria : Springer. 1986. v. 134 (2/3) p. 67-72. ill.
104.- AU: DeHertogh,-A.
TI: Commercial production of Amaryllis (Hippeastrum) potted plants.
SO: Can-Florist-Greenhouse-Nursery. Mississauga, Ont. : Horticulture Publications, Ltd. Sept 1985. v. 80 (9) p. 38, 41. ill.
105.- AU: Conijn,-C.G.M.
TI: New fungicide for the control of Stagonospora in Amaryllis [Hippeastrum, mold, Stagonospora curtisii, Netherlands]. Nieuw stagonospora-bestrijdingsmiddel in Amaryllis.
SO: Vakbl-Bloemisterij. Den Haag, Netherlands : C. Misset. Apr 22, 1983. v. 38 (16) p. 29. ill.
106.- AU: Meerow,-A.W.; Svenson,-S.E.; Kane,-M.E.

TI: DCPTA suppresses growth and flowering of amaryllis.
SO: HortScience. Alexandria, Va. : The American Society for Horticultural Science. Oct 1994. v. 29 (10) p. 1149-1150.

107.- AU: Meerow,-A.W.
TI: New trends in amaryllis (Hippeastrum) breeding.
SO: Proc-Annu-Meet-Fla-State-Hortic-Soc. [S.l.] : The Society. 1988 (pub. May 1989). v. 101 p. 285-288. ill.

108.- AU: Doran,-J.L.
TI: Hippeastrum (Amaryllis) growing.
SO: Herbertia. Irvine, Calif. : International Bulb Society. 1991. v. 47 (1/2) p. 138-144.

109.- AU: Leeuwen,-A.J.M.-van; Buschman,-J.C.M.
TI: The hippeastrum (Amaryllis) as a cut flower.
SO: Herbertia. Irvine, Calif. : International Bulb Society. 1991. v. 47 (1/2) p. 93-102.

110.- AU: Beckham,-E.M.
TI: Hybridizing double Hippeastrum (Amaryllis).
SO: Herbertia. Irvine, Calif. : International Bulb Society. 1991. v. 47 (1/2) p. 56-57.

111.- AU: De-Hertogh,-A.A.; Gallitano,-L.B.
TI: Influence of bulb packing systems on forcing of Dutch-grown Hippeastrum (Amaryllis) as flowering potted plants in North America.
SO: HortTechnology. Alexandria, VA : American Society for Horticultural Science, c1991-. Apr/June 1998. v. 8 (2) p. 175-179.

112.- AU:
TI: Vegetative propagation of Amaryllis
SO: Herbertia, 1: 75-82

113.- AU:
TI: Propagation of Hibrid Amaryllis (Hippeastrum) by cuttage
SO: Science, 78: 532-536

Translocation of potassium and phosphate from ordinary and proteoid roots to shoots in the Proteaceae

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Investigation of potassium and phosphate uptake, using intact plants, showed an accumulation of these elements in proteoid roots, while translocation occurred more readily from ordinary roots. These results, as well as auto-radiographic studies, indicated that proteoid roots may act as sinks. The presence of sucrose in the experimental solution stimulated the translocation of phosphate from the proteoid roots. The inhibition of phosphate translocation by the respiratory uncoupler, 2,4-dinitrophenol (DNP) seems to be evidence for the involvement of an energy dependent mechanism or mechanisms in the translocation of ions from proteoid roots to the aerial parts of the plant.
S. Afr. J. Bot. 1986, 52: 282 – 285

Die bestudering van kalium- en fosfaatopname met intakte plante het aangetoon dat ophoping van hierdie elemente in proteoïede wortels plaasvind, terwyl dit meer geredelik vanuit gewone wortels na die bogrondse dele vervoer word. Hierdie bevindings, sowel as dié verkry tydens outo-radiografiese ondersoeke, dui daarop dat proteoïede wortels as 'n ophopingsgebied ('sink') vir ione dien. Die teenwoordigheid van sukrose in die eksperimentele oplossing bevorder die vervoer van fosfaat vanuit proteoïede wortels. Die onderdrukking van die vervoer deur die respiratoriese ontkoppelaar, 2,4-dinitrofenol (DNP), dui moontlik daarop dat 'n energie-afhanklike meganisme of meganismes in die vervoer van ione vanuit proteoïede wortels na die bogrondse dele betrokke is.
S.-Afr. Tydskr. Plantk. 1986, 52: 282 – 285

Keywords: Phosphate, potassium, proteoid roots

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Introduction

In previous work on potassium and phosphate absorption by ordinary and proteoid roots of the Proteaceae, excised tissues were used (Vorster & Jooste 1986). Since results obtained with excised plant material cannot unconditionally be applied to the whole plant (Lüttge & Higinbotham 1979), the uptake and transport capacities of ordinary and proteoid roots, using intact plants, were investigated.

Materials and Methods

Leucadendron uliginosum R.Br. plants (8 to 12 months old), obtained from nurseries, were used. Plants of comparable size were selected, the roots washed in running tap water, rinsed in deionized water, and subsequently placed in an aerated 0,5 mmol dm^{-3} $CaSO_4$ solution.

Plants were divided into two groups. For absorption by proteoid roots, clusters were immersed separately, but still intact, in the experimental solution. For ordinary roots, a group of roots was placed in the same way in the experimental solution. Figure 1 illustrates the apparatus and the method according to which either the ordinary or the proteoid roots were subjected to absorption of the particular element.

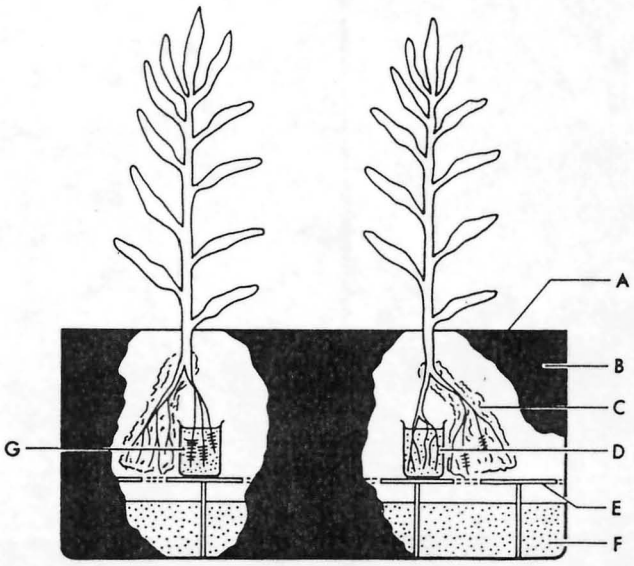

Figure 1 Experimental design to expose intact ordinary or proteoid roots to labelled elements. A. Plastic covering; B. Black paper covering; C. Moistened absorbent paper; D. Ordinary roots exposed to experimental solution; E. Grid; F. Water; G. Proteoid roots exposed to experimental solution.

A large rectangular glass container (300 × 300 × 400 mm) was covered with black paper to avoid exposure of the roots to light. A stainless steel grid was placed at a height of approximately 120 mm from the bottom of the container on which the beakers, containing the experimental solutions, were placed. The container was subsequently filled with water to a level just below the grid to provide a humid atmosphere to the roots not exposed to the experimental solution. Possible desiccation of these roots was further prevented by covering them with moistened absorbent paper. Finally the container was covered with black plastic material.

The roots were exposed to the experimental solution for 24 h, excised, the excess solution was removed by blotting, and the fresh mass determined. The aerial parts were removed and dried in a plant press at 80°C for 12 h. The dried parts were divided into segments (of uniform length in each experiment; segment one just above the root system), and ground separately. Samples of known mass were dry ashed according to an adaptation of the method described by Du Preez *et al.* (1981) and analysed radiometrically by liquid scintillation counting using a commercial scintillation mixture. Potassium and phosphate uptake were calculated from the ^{86}Rb and ^{32}P content of the samples and the specific activity of the experimental solutions, and expressed as μg K (or P) g^{-1} (dry mass of aerial parts) g^{-1} (fresh mass of roots exposed to the experimental solution).

The experimental solutions contained KCl or KH_2PO_4 at a concentration of 0,5 mmol dm^{-3} in a 0,5 mmol dm^{-3} $CaSO_4$ solution, while ^{86}Rb as substitute for ^{42}K (Epstein & Hagen 1952; Epstein 1961; Rains *et al.* 1964) or ^{32}P (both obtained from the Radiochemical Centre, Amersham, U.K.) were added as tracers. Approximately 83,25 kBq per 500 cm^3 experimental solution were used. Amounts of 60 cm^3 of the experimental solutions in 80 cm^3 beakers were used for exposure of the roots.

In the experiments on the effect of sucrose on the translocation of phosphate from proteoid and ordinary roots, the experimental solution contained 200 mmol dm^{-3} sucrose.

In the experiments on the effect of sucrose vs. sucrose plus 2,4-dinitrophenol (DNP) on translocation, only proteoid roots were exposed to the experimental solutions. Furthermore, all the experimental plants in this experiment were previously exposed to a labelled 0,5 mmol dm^{-3} KH_2PO_4 solution (the 'first' solution) for 1 h to ensure absorption of the labelled element — known as a period of pre-loading (Epstein 1972). Hereafter the plants were removed from the first solution and placed in the next experimental solution (the 'second' solution) for a period of 24 h. The second solution was of the same composition as the first solution, but also contained 200 mmol dm^{-3} sucrose, and the tracer was omitted. Half of the series of second solutions contained DNP at a concentration of 0,5 mmol dm^{-3}.

For the autoradiographic investigation, the roots of the plants were rinsed and temporarily placed in an aerated 0,5 mmol dm^{-3} $CaSO_4$ solution at 25°C. The plants were then placed in an aerated experimental solution (500 cm^3) at 25°C for 80 min so that only the roots were covered. The experimental solution contained 0,5 mmol dm^{-3} KH_2PO_4. As in the previous experiments, ^{32}P (166,5 kBq per 500 cm^3) was used as tracer.

Three to four replicates of each treatment were employed; each experiment was repeated at least twice on consecutive days. The mean and standard error for each treatment were calculated. Differences between means of more than twice the standard error were regarded as significant.

Results and Discussion

Translocation of potassium and phosphate from ordinary and proteoid roots to the shoot

The results presented in Tables 1 and 2 might appear to be contradictory to previous findings, namely that proteoid roots are characterized by a higher absorption capacity than ordinary roots (Vorster & Jooste 1986). Repeated execution of the experiment confirmed that the elements concerned are translocated much more effectively from the ordinary roots than from the proteiod roots to the aerial parts. Approximately 278 and 168% more potassium and phosphate respectively were translocated from the ordinary than from the proteoid roots to the shoot.

This observation could have been influenced by the initial potassium and phosphate status of the roots. If it is assumed that the proteoid roots initially had a higher internal potassium and phosphate content than ordinary roots, and that the incoming label equilibrates at least partially with the internal pools of potassium and phosphate, this could cause a differential isotopic dilution of the incoming label, with the effect that label entering proteoid roots becomes more heavily diluted (internal label has a lower specific activity in proteoid roots). Consequently a greater amount of label could have been present in the shoots of plants to which potassium and phosphate were supplied to the ordinary roots.

In view of previous work (Vorster & Jooste 1986) a significantly higher initial potassium and phosphate content of proteoid roots seems unlikely, and proteoid roots probably serve as a temporary sink. This possibility was also suggested by Jeffrey (1967), Gardner *et al.* (1981), and Specht (pers. comm.).

Table 1 Uptake (± standard error) and distribution of potassium in shoots following exposure of ordinary and proteoid roots to a 0,5 mmol dm^{-3} KCl solution

	K uptake[a]	
Shoot segments	Proteoid roots exposed	Ordinary roots exposed
1	12,15 ± 2,85	52,63 ± 6,28
2	2,84 ± 0,48	8,31 ± 0,79
3	1,36 ± 0,48	4,02 ± 0,89
4	1,80 ± 0,23	3,59 ± 0,91

[a]μg K g^{-1} (dry mass of aerial parts) g^{-1} (fresh mass of exposed roots)

Table 2 Uptake (± standard error) and distribution of phosphate in shoots following exposure of ordinary and proteoid roots to a 0,5 mmol dm^{-3} KH_2PO_4 solution

	P uptake[a]	
Shoot segments	Proteoid roots exposed	Ordinary roots exposed
1	35,0 ± 12,15	72,8 ± 17,38
2	26,8 ± 8,21	79,3 ± 19,82
3	23,6 ± 7,61	86,1 ± 11,00
4	25,2 ± 9,66	74,0 ± 17,31
5	31,1 ± 8,50	63,0 ± 12,08
6	10,1 ± 3,74	31,4 ± 7,68

[a]μg P g^{-1} (dry mass of aerial parts) g^{-1} (fresh mass of exposed roots)

Autoradiographic investigation

In view of the above findings, it was decided to try to gain more insight into the uptake by and translocation from ordinary and proteoid roots by means of an autoradiographic investigation.

Figure 2 clearly shows an accumulation of ^{32}P in the proteoid roots. Even where the ordinary roots show a dense cluster on the photograph the activity is not as high as in the proteoid roots.

These results further support the assumption that the proteoid roots accumulate phosphate while it is more readily translocated from ordinary roots to the shoot.

The effect of sucrose on the translocation of phosphate from ordinary and proteoid roots to the shoot

In view of previous findings showing that proteoid roots possess a greater capacity for metabolic absorption than ordinary roots (Vorster & Jooste 1986), the possibility exists that the lack of a source of sufficient energy may restrict translocation to the aerial parts of the plant.

It was found that sucrose was essential in the ambient solution to maintain continuous xylem sap exudation in excised maize roots (Jooste unpublished data). Gorham (pers. comm.) confirmed this finding. In view of this it was decided to establish whether the presence of sucrose in the experimental solution influences the translocation of ions from proteoid and ordinary roots to the shoot.

In the absence of sucrose (Table 2), about 168% more phosphate was translocated from ordinary roots than from proteoid roots. In contrast to this, in the presence of sucrose (Table 3), in total approximately 18% more phosphate was translocated from proteoid than from ordinary roots to the aerial parts of the plants.

From these results it is clear that sucrose must have contributed to the translocation of phosphate from the proteoid roots. It is possible that an energy dependent mechanism, or mechanisms, might be involved in the translocation of ions from proteoid roots to the shoot.

The effect of sucrose versus sucrose plus DNP on the translocation of phosphate from proteoid roots to the shoot

In view of the enhanced ion translocation by proteoid roots in the presence of sucrose, the possible involvement of an energy yielding process might be suspected. Eliminating such a process by addition of DNP, a respiratory uncoupler, would thus shed additional light on this phenomenon. Results obtained in this experiment are presented in Table 4.

Table 3 Uptake (± standard error) and distribution of phosphate in shoots following exposure of ordinary and proteoid roots to a 0,5 mmol dm^{-3} KH_2PO_4 solution plus sucrose

	P uptake[a]	
Shoot segments	Proteoid roots exposed	Ordinary roots exposed
1	81,12 ± 10,92	64,76 ± 12,42
2	21,64 ± 3,70	25,45 ± 9,47
3	13,62 ± 6,97	7,89 ± 3,06
4	7,05 ± 5,36	6,11 ± 3,29

[a] $\mu g\ P\ g^{-1}$ (dry mass of aerial parts) g^{-1} (fresh mass of exposed roots)

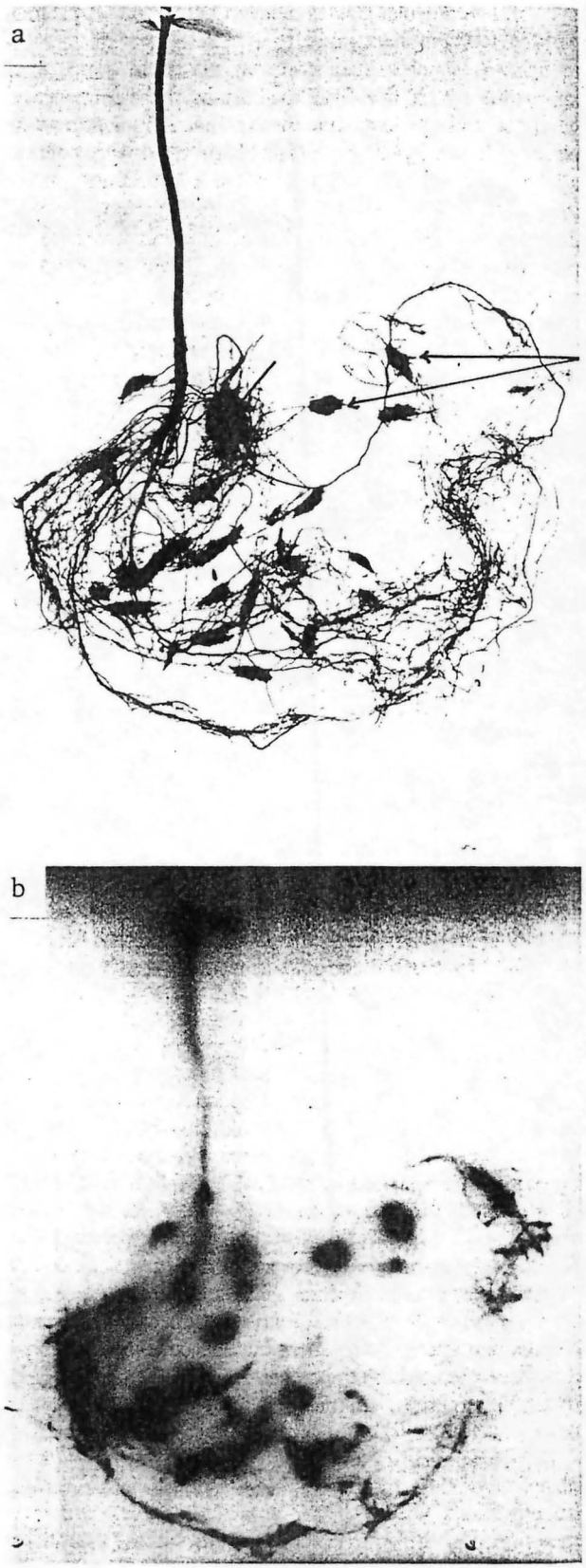

Figure 2 Photograph (a) and autoradiograph (b) of root system following exposure to a labelled 0,5 mmol dm^{-3} KH_2PO_4 solution. (Arrows indicate proteoid roots.)

DNP inhibited phosphate translocation to the shoot by approximately 80%, presumably by suppressing ATP-synthesis, making less energy available for the translocation of phosphate to the aerial parts of the plants.

Table 4 Uptake (± standard error) and distribution of phosphate in shoots following exposure of proteoid roots to a 0,5 mmol dm^{-3} KH_2PO_4 solution (plus sucrose) with and without DNP

Shoot segments	P uptake[a]	
	With DNP	Without DNP
1	4,32 ± 2,56	28,47 ± 5,55
2	2,03 ± 2,48	5,81 ± 1,93
3	1,57 ± 1,92	3,67 ± 4,64
4	0,62 ± 0,19	1,68 ± 0,67

[a]µg P g^{-1} (dry mass of aerial parts) g^{-1} (fresh mass of exposed roots)

The amount of potassium (Table 1) and phosphate (Tables 2,3 & 4) in the different shoot segments show that both elements are quite readily distributed throughout the aerial parts of the plants.

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References

DU PREEZ, M., CARSTENS, J. & VAN WYK, E. 1981. Voorbereiding en droogverassing van blaarmonsters vir ontleding. N.I.V.V.-prosedure en tegnieke Nr. 32, N.I.V.V., Stellenbosch.

EPSTEIN, E. 1961. The essential role of calcium in selective cation transport by plant cells. *Pl. Physiol.* 36: 437 – 444.

EPSTEIN, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Inc., New York.

EPSTEIN, E. & HAGEN, C.E. 1952. A kinetic study of the absorption of alkali cations by barley roots. *Pl. Physiol.* 27: 457 – 474.

GARDNER, W.K., PARBERRY, D.G. & BARBER, D.A. 1981. Proteoid root morphology and function in *Lupinus albus*. *Pl. Soil* 60: 143 – 147.

JEFFREY, D.W. 1967. Phosphate nutrition of Australian heath plants. 1. The importance of proteoid roots in *Banksia* (Proteaceae). *Aust. J. Bot.* 15: 403 – 411.

LÜTTGE, U. & HIGINBOTHAM, N. 1979. Transport in plants. Springer-Verlag, New York.

RAINS, D.W., SCHMID, W.E. & EPSTEIN, E. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. *Pl. Physiol.* 39: 274 – 278.

VORSTER, P.W. & JOOSTE, J.H. 1986. Potassium and phosphate absorption by excised ordinary and proteoid roots of the Proteaceae. *S. Afr. J. Bot.* 52: 277 – 281.

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Effects of nutrients on the distribution of dry mass, nitrogen and phosphorus in seedlings of *Protea repens* (L.) L. (Proteaceae)

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SUMMARY

The response of seedlings of the sclerophyllous shrub, *Protea repens* (L.) L., to increasing concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrients excluding N and P (M) was determined in potted Clovelly soil collected from a lowland fynbos site at Pella, south-western Cape, South Africa. Pot culture resulted in increased soil mineral nitrogen, in particular nitrate, and decreased available (resin-extractable) phosphorus concentrations compared to field soil. High amounts of N (4–64 g m^{-2}N) and M addition resulted in seedling mortality. Plant dry mass, leaf area and phosphorus and nitrogen contents increased in response to increasing application of P, but no significant differences were found in response to M. Increasing applications of N resulted in reduced plant dry mass, leaf area and phosphorus content. These patterns of mortality and growth are interpreted as a response to an imbalance between nitrogen and phosphorus availability. These results are compared to the response of mature fynbos shrubs to fertilizer additions in the field and the responses of pot-grown sclerophyllous plants from other Mediterranean-type ecosystems.

Key words: Proteaceae, allocation patterns, nutrient additions, mineral nutrition, root/shoot ratios.

INTRODUCTION

Protea repens (L.) L. is a common and widespread species in mountain and lowland fynbos vegetation of the south-western Cape, South Africa, in contrast to most other Cape species of the Proteaceae which are more restricted in their distributions. It is a slow growing evergreen shrub of 1–4 m in height and is prevalent in the late stages of fynbos succession. Its large seeds are mainly released from the flowerheads after the parent plants have been exposed to fire (Bond, 1985) and contain high nutrient reserves, particularly nitrogen and phosphorus (Pate *et al.*, 1986), which allow initial seedling growth to be largely independent of external supplies of nutrients. When the nutrient reserves in the cotyledons are almost depleted, nutrient uptake from the soils of low nutrient status is facilitated by proteoid roots (Lamont, 1982). In addition, *P. repens* has a low rate of NO_3^- and NH_4^+ assimilation and a low rate of nitrogen metabolism (Stock & Lewis, 1984).

The aim of this study was to examine the effects of a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrients excluding N and P (M) on the distribution of dry mass, phosphorus and nitrogen in seedlings of *P. repens*. Comparisons of the concentrations of plant-available forms of phosphorus and nitrogen between soils in the pots and the field site from where the potted soil was collected were undertaken to establish whether the results of this experiment could be extrapolated to the field.

METHODS

Plant growth

Seeds were germinated by immersion in 10% H_2O_2 for 6 h and then planted into asbestos trays (10 cm depth) containing an autoclaved (121 °C for 15 min) mixture of 50% 2 mm sieved Clovelly soil and 50% acid-washed sand on 1st October 1984. The Clovelly soil (orthic A horizon overlying a yellow-brown apedal B) was collected from Pella (0–20 cm depth), a sand-plain lowland fynbos site, 62 km north of Cape Town on the western coastal forelands, which

has an annual rainfall of 522 mm per annum. This soil is of a low phosphorus and nitrogen status and consists predominantly of medium textured sand (Mitchell, Brown & Jongens-Roberts, 1984; Stock & Lewis, 1986). The trays were incubated at 0 °C for 4 days and then transferred to a well-ventilated glasshouse at the Botany Department, University of Cape Town. Seeds germinated after 3 weeks (55 % germination). Each seedling was transplanted after a further 5 weeks into plastic bags (surface area of 270 cm^2 and depth of 30 cm) containing Clovelly sand and grown in the glasshouse until June 1985 when at the age of 8 months the cotyledons were chlorotic as the reserves were depleted. In the glasshouse, midday light intensities on clear days ranged from 800–1500 μmol photons $m^{-2} s^{-1}$, temperatures ranged from 10–32 °C and relative humidity from 50–95 %. Plants were watered with deionized water to saturation at 3 day intervals.

Nutrient applications

Seedlings were amended only once with one of three treatments, applied randomly at various concentrations on 14 June 1985 and replicated five times:

1. (P) Phosphorus ($Ca_3(PO_4)_2$) at 0, 0·2, 0·4, 0·8, 1·6, 3·2, 6·4 and 12·8 g m^{-2} P.

2. (N) Nitrogen (NH_4NO_3), at 0, 2, 4, 8, 16, 32 and 64 g m^{-2} N, (based on the N:P ratio of a Long Ashton nutrient solution which is 10; Hewitt & Smith, 1975).

3. (M) A mixture of all essential nutrients excluding N and P based on a Long Ashton nutrient solution (Hewitt & Smith, 1975) in proportion to the N and P additions. It consisted of K_2SO_4, $MgSO_4$, $CaCl_2$, $NaCl_2$, Fe citrate.$5H_2O$, $MnSO_4.4H_2O$, H_3BO_3, $ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$ (e.g. K additions of 0, 0·76, 1·52, 3·04, 6·09, 12·18 and 24·35 g m^{-2} K).

Plant harvesting and nutrient analyses

Plants were harvested in February 1986, 8 months after the nutrient applications, by which time they were 16 months old. Plant height, leaf number and the proportion of necrotic, partially necrotic and abscissed leaves were determined prior to harvesting. Five fully expanded leaves were removed from each seedling, leaf area determined and then oven-dried for determination of leaf specific mass. The soil was removed from the root system by wet sieving and the leaves, the stem and root system immediately separated to prevent the translocations of nutrients between plant parts after harvesting. These were oven-dried at 80 °C for 48 h and weighed. Plant material was ground to 40 mesh using a Wiley mill and phosphorus and nitrogen determined on at least two replicates of each sample, using the same analytical methods as Witkowski & Mitchell (1987).

Soil analyses

Soil samples were taken from the pots (0–5 cm depth) 21, 96 and 171 days after fertilizer addition as well as from the field site (0–20 cm depth). All soils were analysed for resin-extractable and Bray No. 2 phosphorus, ammonium and nitrate. Total nitrogen, total phosphorus, organic matter and pH were determined on the potted soil samples taken 21 and 171 days after fertilizer addition and from the field site. The soil analytical methods were the same as those used in Mitchell *et al.* (1984) and Stock & Lewis (1986).

Statistical analyses

Statistical analyses were performed separately for each treatment. Comparisons between potted soils were analysed by analysis of variance with repeated measures and between unfertilized field and pot samples by *t* tests. Soil mineral nutrient concentrations were $\log_{10}(X+1)$ transformed and all percentage values were arcsin transformed prior to statistical analysis. Plant responses to nutrient applications were determined by regression analyses. Both the independent and the dependent variables were $\log_{10}(X+1)$ transformed.

RESULTS

Soil responses

A decrease in ammonium and resin-extractable phosphorus concentrations and an increase in nitrate concentration were found in unfertilized, 8-month-old potted soil supporting *P. repens*, compared to field soil (Table 1). Soil organic matter, pH, total nitrogen and total phosphorus remained unchanged (Table 1). After the application of nutrients to the pots, an increase in soil pH with time and with increasing P application level (from pH 5·2 to 5·6 at 12·8 g m^{-2} P) and a decrease with N application level (from pH 5·2 to 5·0 at high N application levels of 32–64 g m^{-2} N) were found. Soil organic matter decreased with incubation time, except with M application, where it increased from 0·9 % in unfertilized pots to 1·4 % at high M application levels. There were rapid decreases in soil ammonium and nitrate concentrations with time in the N amended pots (Fig. 1). The nitrate to ammonium ratio and total mineral nitrogen concentration in unfertilized pot incubated soils were approximately one order of magnitude higher than found in the field (Fig. 1, Table 1). Total nitrogen concentrations decreased with time in the N amended pots (Fig. 1). No significant differences in total and Bray No. 2 phosphorus concentrations were found in the P amended pots with incubation time, whereas resin-extractable phosphorus concentrations declined (Fig. 2).

Table 1. *Comparison of the chemical properties of unfertilized Clovelly soil (mean) in the field and after 8 months in a glasshouse pot experiment supporting* Protea repens *seedlings.*

	Field	Pot
Organic matter (%)	0·84	0·89
pH	5·3	5·2
Ammonium (μg N g^{-1} dry mass)	1·2	0·6**
Nitrate (μg N g^{-1} dry mass)	0·5	6·8*
Resin-extractable-P (μg P g^{-1} dry mass)	0·3	0·1**
Bray No. 2-P (μg P g^{-1} dry mass)	2·6	3·3
Total nitrogen (μg N g^{-1} dry mass)	245	276
Total phosphorus (μg P g^{-1} dry mass)	28·5	29·5

* Significant differences by t test, $P < 0{\cdot}05$ (d.f. = 8); ** $P < 0.01$.

Plant responses

Three days after nutrients had been applied, several plants amended with 32 and 64 g m^{-2} N became necrotic and subsequently died. These plants were found to have high nitrogen concentrations, in the range of 46–66 mg g^{-1} dry mass N, compared to a mean and S.E of 16 ± 1 mg g^{-1} dry mass N for unfertilized control plants harvested at the same time. In addition, further plants amended with N (4–64 g m^{-2} N) and some amended with high levels of M became necrotic and some of these subsequently died during November when temperatures were high (Fig. 3). No mortality of *P. repens* seedlings in response to P addition was found (Fig. 3). In the surviving plants, the proportion of normal leaves (those that were not necrotic, partially necrotic or abscissed) per plant, was significantly lower with increasing N and higher with increasing P application levels ($P < 0{\cdot}05$). No significant trends in plant height and leaf number per plant in response to

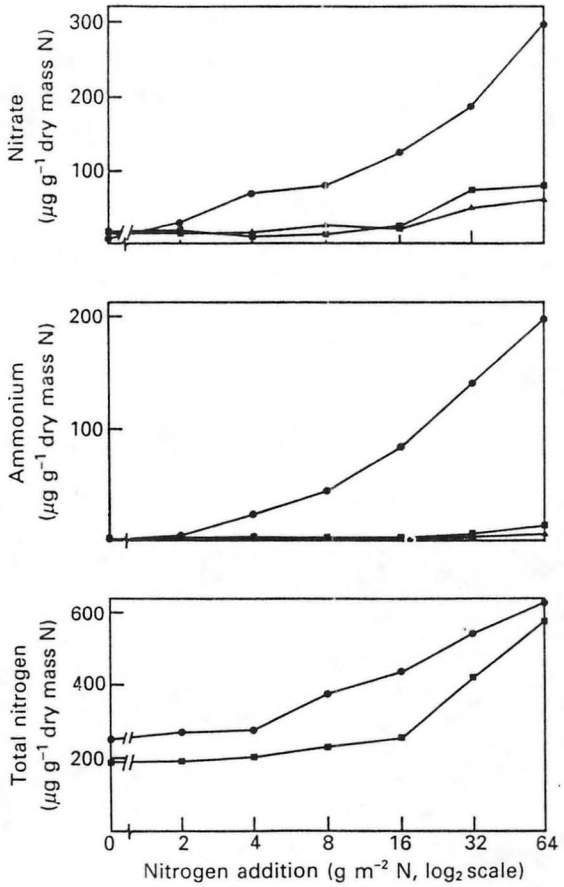

Figure 1. Nitrate, ammonium and total nitrogen concentrations 21 (●), 96 (▲) and 171 (■) days after amendment with a range of nitrogen concentrations in potted Clovelly soil supporting *Protea repens* seedlings. Each point is the mean of five replicates.

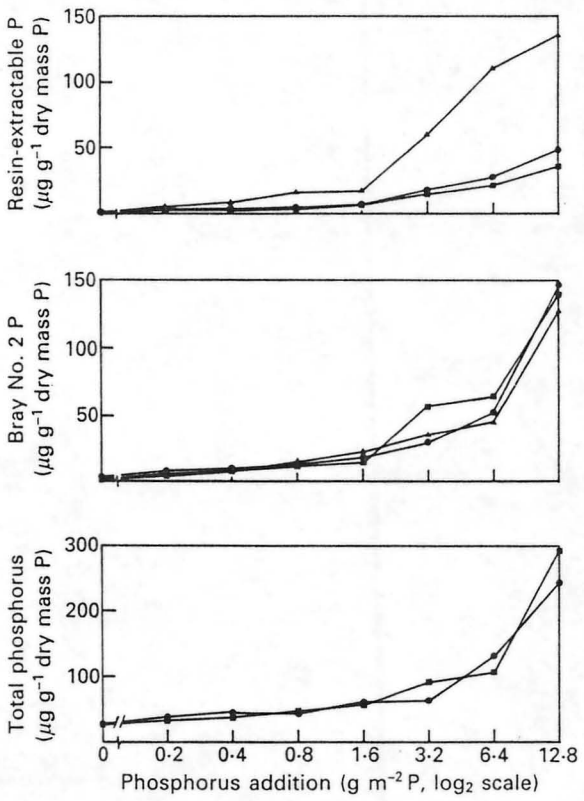

Figure 2. Resin-extractable, Bray No. 2 and total phosphorus concentrations 21 (●), 96 (▲) and 171 (■) days after amendment with a range of phosphorus concentrations in potted Clovelly soil supporting *Protea repens* seedlings. Each point is the mean of five replicates.

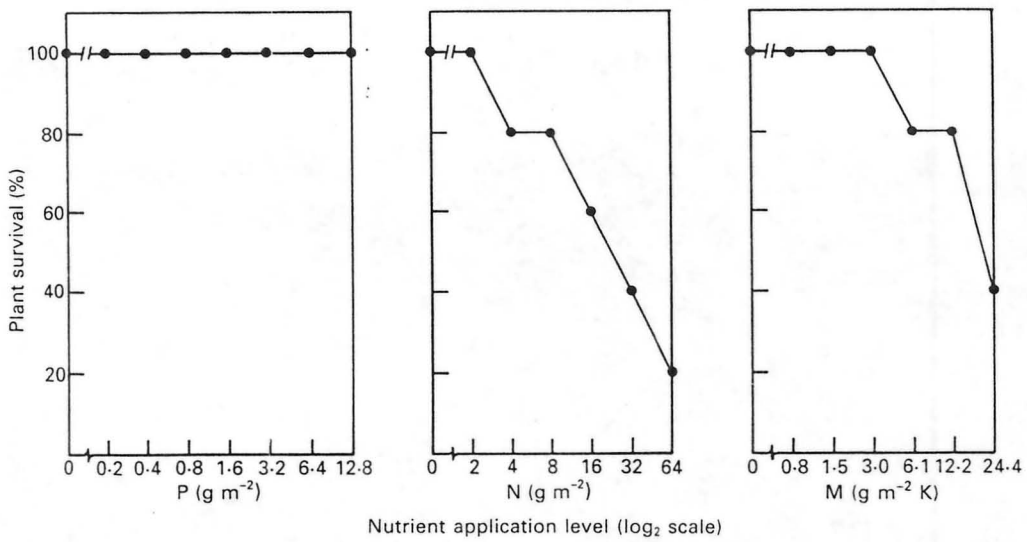

Figure 3. Survival of 16-month-old *Protea repens* seedlings after 8 months growth in a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrient excluding N and P (M). Additions of P, N and M are in the proportions found in a Long Ashton nutrient solution (Hewitt & Smith, 1975) with M presented as K additions.

variation in nutrient application level were found for any of the treatments.

Leaf, stem and total plant dry mass increased significantly with increasing P application level ($P < 0{\cdot}01$; Fig. 4) whereas increasing N application resulted in reduced total plant dry mass ($P < 0{\cdot}01$; Fig. 4). Leaf, stem, root and total plant phosphorus contents increased in response to increasing P application level ($P < 0{\cdot}001$) and decreased in response to that of N ($P < 0{\cdot}05$; Fig. 5). Total plant, leaf and stem nitrogen contents increased with increasing P application level ($P < 0{\cdot}05$), while root nitrogen content decreased with increasing N application level ($P < 0{\cdot}05$, Fig. 6). No significant differences in dry mass, phosphorus and nitrogen contents were found in response to the application of M (Fig. 4). Leaf specific mass tended to decrease with increasing levels of application of all three treatments (Fig. 7). However, plant leaf area increased with increasing P application level ($P < 0{\cdot}001$) and decreased with that of N ($P < 0{\cdot}05$; Fig. 7).

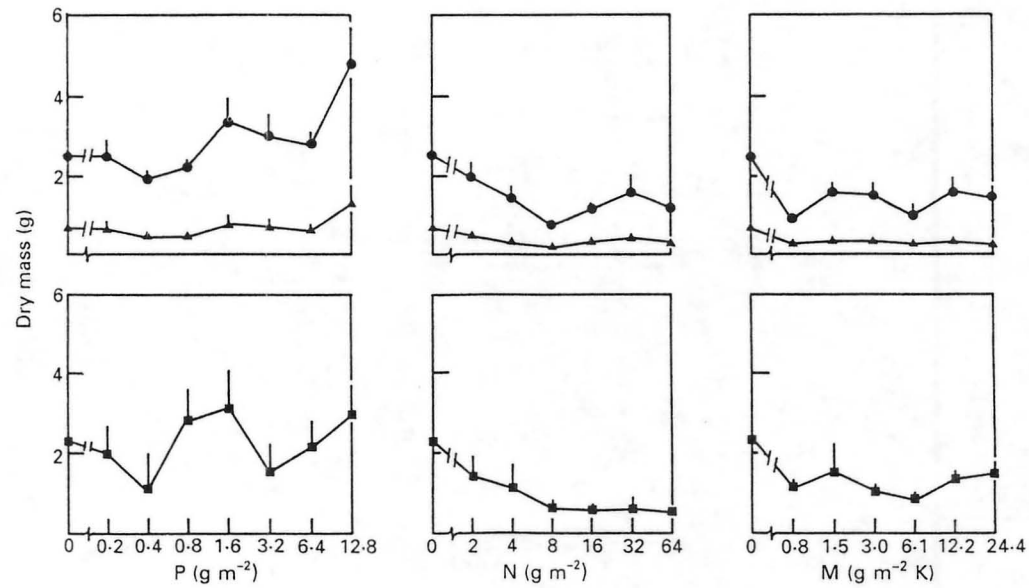

Figure 4. Dry mass of *Protea repens* seedlings grown in a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrients excluding N and P (M) in potted Clovelly soil. Additions of M are presented as K addition. Symbols: ●, leaf; ▲, stem; ■, root. Vertical bars represent SE.

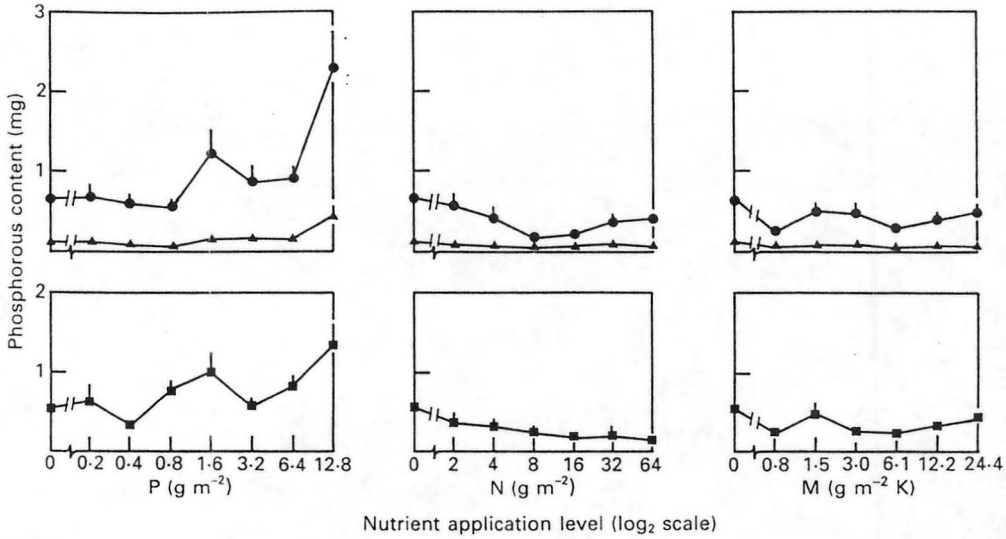

Figure 5. Phosphorus content of *Protea repens* seedlings grown in a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrient excluding N and P (M) in potted Clovelly soil. Additions of M are presented as K additions. Symbols: ●, leaf; ▲, stem; ■, root. Vertical bars represent SE.

The root-to-shoot ratio of dry mass decreased significantly with increasing application levels of both N ($P < 0{\cdot}001$) and P ($P < 0{\cdot}05$). Root-to-shoot nitrogen ratio increased with increasing N ($P < 0{\cdot}01$) and M ($P < 0{\cdot}05$) application levels, whereas no significant trends in root-to-shoot phosphorus ratio were found with increasing application levels of any of the three treatments.

DISCUSSION

This study has shown that the growth of *P. repens* seedlings in Clovelly soil pot culture is stimulated by phosphorus additions of 0·2–12·8 g m^{-2} P, whereas additions of 4–64 g m^{-2} N and high M additions resulted in seedling mortality and reduced growth. In a field fertilizer experiment, 4- to 5-year-old shrubs of *Leucospermum parile* (Proteaceae) and *Phylica cephalantha* (Rhamnaceae), growing in the same Clovelly soil, responded to a factorial fertilizer addition, of the same three treatments applied in this study (0·5 g m^{-2} P, 5 g m^{-2} N and M addition represented by 1·9 g m^{-2} K), with increases in shoot growth of both species in response to nitrogen and not phosphorus addition (Witkowski, 1988). The different responses between potted *P. repens* and the

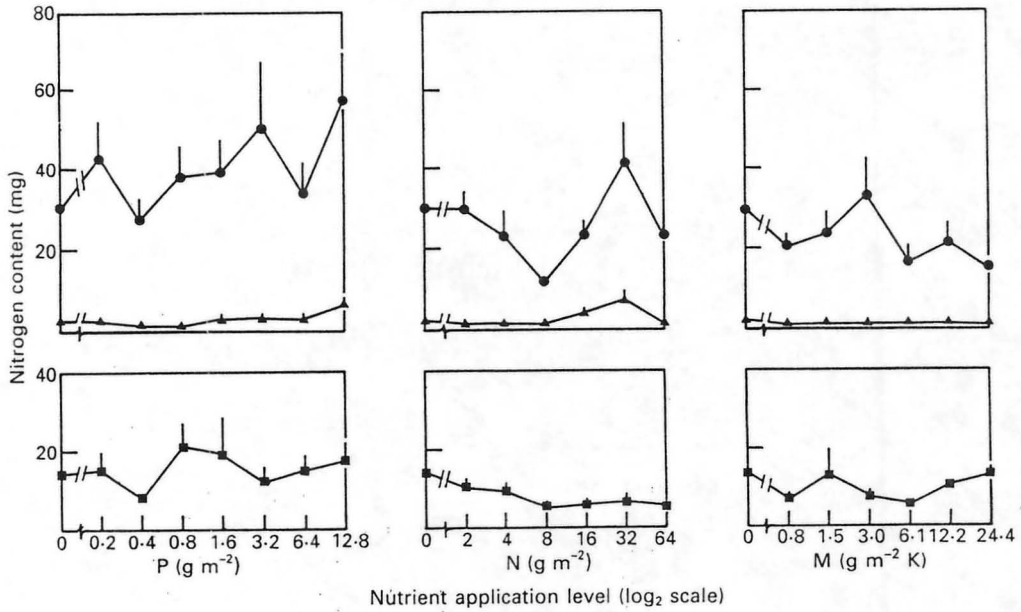

Figure 6. Nitrogen content of *Protea repens* seedlings grown in a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrient excluding N and P (M) in potted Clovelly soil. Additions of M are presented as K additions. Symbols: ●, leaf; ▲, stem; ■, root. Vertical bars represent SE.

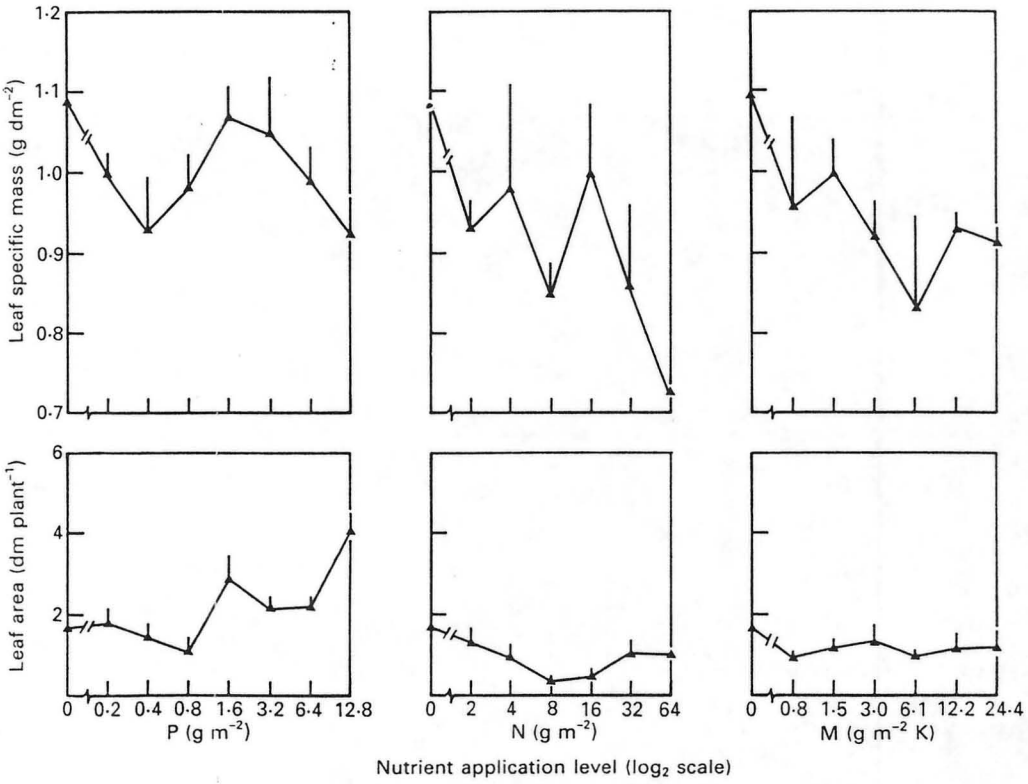

Figure 7. Leaf area and leaf specific mass of *Protea repens* seedlings grown in a range of concentrations of phosphorus (P), nitrogen (N) and a mixture of all essential nutrient excluding N and P (M) in potted Clovelly soil. Additions of M are presented as K additions. Vertical bars represent SE.

shrubs at the field site can be explained by differences in nutrient availability between potted and field soils. Unfertilized potted soil exhibited greater concentrations of mineral nitrogen, particularly nitrate, and reduced concentrations of resin-extractable phosphorus, compared with field soil. Incubation studies on this soil have shown that relatively high soil moisture contents stimulated nitrogen mineralization (Stock, Lewis & Allsopp, 1988).

Average moisture availability is higher in the pots than at the field site (Moll & Sommerville, 1985). Although moderate soil moisture contents of 9–18 % of field capacity enhanced phosphorus mineralization, incubation at 50–100 % of field capacity resulted in reduced concentrations of resin-extractable phosphorus (Baker & Witkowski, unpublished). Thus the ratio of available nitrogen to phosphorus is increased by approximately one order of magnitude in potted soil compared with that at the field site. Similar results were found by Kachi & Hirose (1983) for coastal sand dune soil in Japan, suggesting that the ratio of plant available nitrogen to phosphorus may be at least as important, in determining the range of a species, as the absolute amounts of available N and P. The reduced growth of surviving *P. repens* seedlings in response to increasing levels of application of N, and to a lesser extent M, appears to be the result of an imbalance between nitrogen and phosphorus availability. The increase in the availability of a nutrient may result in a decrease in the relative availability of other nutrients (Lajtha & Klein, 1988). Although plants can compensate, to a large degree, for imbalances in the availability of resources (Chapin *et al.*, 1987), the addition of high levels of N to the pots resulted in a still greater nutrient imbalance, leading to toxicity and plant mortality.

In Australian heathlands, which are edaphically similar to those of the fynbos biome (Witkowski & Mitchell, 1987), phosphorus fertilizer addition resulted in reduced seedling survival, but increased growth in both surviving seedlings and mature heath plants (Specht, 1963). Pot culture of three *Banksia* spp. (Proteaceae; Siddiqi, Myerscough & Carolin, 1976) and other heath species (Grundon, 1972; Specht & Groves, 1966) in P-amended heathland soil, resulted in reduced growth and the appearance of phosphorus toxicity symptoms. A similar study of three sclerophylls showed mortality of seedlings at high levels of application of both N and P (Groves & Keraitis, 1976), with P toxicity symptoms usually associated with leaf P concentrations of 0·8 % and above (Ozanne & Specht, 1981). In this study on *P. repens*, leaf phosphorus concentrations of only 0·06 % were found with the addition of 12·8 g m^{-2} P. Phosphorus toxicity is alleviated by supply of N and K (Grundon, 1972) and K (Siddiqi *et al.*, 1976),

indicating that a nutrient imbalance may again be involved.

Increasing levels of application of both N and P resulted in a trend of decreasing root-to-shoot dry mass ratio. Similar compensatory growth has been found in response to imposed nutrient and moisture stress in graminoids and other herbaceous plants (Davidson, 1969; Hunt & Nicholls, 1986). Similarly, an increase in root to shoot ratio in response to the addition of M (1·9 g m^{-2} K) and a decrease in response to N (5 g m^{-2} N) were found in 2-year old *Thamnochortus punctatus* (Restionaceae) plants growing in Clovelly soil at the Pella field site (Witkowski, 1988). Although *P. repens* is a widely distributed species and has a broader ecological niche than most other members of the Proteaceae in the fynbos biome, the degree of response of these seedlings to nutrient additions is low compared with that found in graminoid species (Davidson, 1969; Hunt & Nicholls, 1986). This study confirms that this species has a conservative response to nutrient additions which corresponds to its slow growth habit and is thus similar to nutrient stressed evergreen shrubs from many parts of the world (Chapin, 1980).

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REFERENCES

Bond, W. J. (1985). Canopy stored seed reserves (serotiny) in Cape Proteaceae. *South African Journal of Botany* **51**, 181–186.

Chapin, F. S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**, 233–260.

Chapin, F. S., Bloom, A. J., Field, C. B. & Waring, R. H. (1987). Plant responses to multiple environmental factors. *Bioscience* **37**, 49–57.

Davidson, R. L. (1969). Effects of soil nutrients and moisture on root/shoot ratios in *Lolium perenne* L. and *Trifolium repens* L. *Annals of Botany* **33**, 571–577.

Groves, R. H. & Keraitis, K. (1976). Survival and growth of seedlings of three sclerophyllous species at high levels of phosphorus and nitrogen. *Australian Journal of Botany* **24**, 681–690.

Grundon, N. J. (1972). Mineral nutrition of some Queenland heath plants. *Journal of Ecology* **60**, 171–181.

Hewitt, E. J. & Smith, T. A. (1975). *Plant Mineral Nutrition*. The English University Press, London.

Hunt, R. & Nicholls, A. O. (1986). Stress and the coarse control of growth and root–shoot partitioning in herbaceous plants. *Oikos* **47**, 149–158.

Kachi, N. & Hirose, T. (1983). Limiting nutrients for plant growth in coastal sand dune soils. *Journals of Ecology* **71**, 937–944.

Lajtha, K. & Klein, M. (1988). The effects of varying nitrogen and phosphorus availability on nutrient use by *Larrea tridentata*, a desert evergreen shrub. *Oecologia* **75**, 348–353.

Lamont, B. B. (1982). Mechanisms for enhancing nutrient uptake in plants, with particular reference to Mediterranean South Africa and Western Australia. *The Botanical Review* **48**, 597–689.

Mitchell, D. T., Brown, G. & Jongens–Roberts, S. M. (1984). Variations of forms of phosphorus in the sandy soils of coastal fynbos, south-western Cape. *Journal of Ecology* **72**, 575–584.

Moll, E. J. & Sommerville, J. E. M. (1985). Seasonal xylem pressure potentials of two South African coastal fynbos species in three soil types. *South African Journal of Botany* **51**, 187–193.

Ozanne, P. G. & Specht, R. L. (1981). Mineral nutrition of heathlands: Phosphorus toxicity. In: *Ecosystems of the World*, vol. 9B, *Heathlands and Related Shrublands* (Ed. by R. L. Specht), pp. 209–213. Elsevier, Amsterdam.

Pate, J. S., Rasins, E., Rullo, J. & Kuo, J. (1986). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. *Annals of Botany* **57**, 747–770.

Siddiqi, M. Y., Myerscough, P. J. & Carolin, R. C. (1976). Studies in the ecology of coastal heath in New South Wales. IV. Seed survival, germination, seedling establishment and early growth in *Banksia serratifolia* Salisb., *B. asplenifolia* Salisb. and *B. ericifolia* L.f. in relation to fire, temperature and nutritional effects. *Australian Journal of Ecology* **1**, 175–183.

Specht, R. L. (1963). Dark Island heath (Ninety-Mile Plain, South Australia). VII. The effects of fertilizers on composition and growth, 1950–1960. *Australian Journal of Botany* **11**, 67–94.

Specht, R. L. & Groves, R. H. (1966). A comparison of the phosphorus nutrition of Australian heath plants and introduced economic plants. *Australian Journal of Botany* **14**, 201–221.

Stock, W. D. & Lewis, O. A. M. (1984). Uptake and assimilation of nitrate and ammonium by an evergreen fynbos shrub species *Protea repens* L. (Proteaceae). *New Phytologist* **97**, 261–268.

Stock, W. D. & Lewis, O. A. M. (1986). Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. *Journal of Ecology* **74**, 317–328.

Stock, W. D., Lewis, O. A. M. & Allsopp, N. (1988). Soil nitrogen mineralization in a coastal fynbos succession. *Plant and Soil* **106**, 295–298.

Witkowski, E. T. F. (1988). *Response of a sand-plain lowland fynbos ecosystem to nutrient additions*. Ph.D. thesis, University of Cape Town.

Witkowski, E. T. F. & Mitchell, D. T. (1987). Variations in soil phosphorus in the fynbos biome, South Africa. *Journal of Ecology* **75**, 1159–1171.

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Effect of sampling time and leaf position on leaf nutrient composition of *Protea* 'Pink Ice'

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Summary. Seasonal fluctuations in the concentrations of 12 nutrients were assessed over 3 years for *Protea* 'Pink Ice' in 3 plantings in the Mount Lofty Ranges of South Australia. Nutrient concentrations in youngest fully expanded leaves (YFEL) generally showed strong seasonal trends, reflecting seasonal vegetative and flowering patterns. During May–August and December–February, YFEL concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sodium (Na), sulfur (S), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) were relatively stable, making these suitable times for sampling.

The effects of sampling error and leaf position were also determined. The error associated with our sampling procedure was within acceptable limits (coefficients of variation <15%) for N, P, K, Ca, magnesium (Mg), Na, S, and boron (B). Differences in nutrient composition between YFEL and YFEL – 1, YFEL – 2, YFEL + 1, YFEL + 2, and YFEL + 3 were of little practical significance.

Nutrient removal by flowering stems and concentrations of nutrients in different fractions (bloom, stem + leaves, axillary shoots) of flowering stems were determined for each site. Nutrient concentrations in flowering stems were generally lower than in leaves. Nitrogen concentrations in axillary shoots and K concentrations in blooms were significantly higher than in other fractions.

For preferred sampling times, seasonal trends showed that concentrations of N, P, K, Ca, Na, S, Cu, and Fe were fairly stable over May–August. Similarly, concentrations of N, P, K, Ca, S, Zn, and Mn were relatively stable during December–February, after completion of the spring vegetative flush.

Introduction

Plant analysis is extensively used to determine the nutrient status of a wide range of annual and perennial agricultural and horticultural crops (Reuter and Robinson 1986). In Australia many studies to develop leaf-sampling procedures and interpretation standards have been published for a wide range of perennial crops including apple (Graley 1982), custard apple (George *et al.* 1989), grapes (Robinson and McCarthy 1985), kiwifruit (Cresswell 1989), macadamia (Stephenson *et al.* 1986), pecan (Cresswell and Wickson 1986), and pome and stone fruit (Leece 1976). These studies showed that 3 factors must be considered when developing plant-sampling procedures for perennial species. Firstly, index tissue should be easily identifiable and able to be sampled in a reproducible manner to minimise sampling error. Secondly, the chemical composition of index tissues, for example leaves, may change with age or position along a shoot. It is therefore important to determine the effect on nutrient composition of sampling leaves next in age to the index leaf. Thirdly, seasonal trends exist in nutrient composition, and the preferred sampling time is when the rate of change in nutrient concentrations is minimal.

Tentative interpretation standards for perennial crops may be established by monitoring the nutrient status of commercial plantings that have the desired productivity and quality characteristics. The standards may be modified with use (Cresswell 1989). For reliable interpretation, the plant-sampling procedure must be carefully followed; environmental and crop management factors, nutrient interactions, plant genotype differences, and rootstock also need to be taken into account (Lewis *et al.* 1993).

No study has been published in Australia on these aspects of the nutrient management of *Protea* species. Cresswell (1991) presented data on the phosphorus (P) nutrition of *Protea* hybrids 'Satin Mink' and 'Pink Ice'. Price (1986) presented plant test interpretation standards for *P. cynaroides*, *P. magnifica*, and *P. repens*. Working in South Africa, Claassens (1981, 1986) studied the effect of supply of nitrogen (N), P, potassium (K), and sodium (Na), and source of N, on the growth and nutrient uptake of a range of *Protea* species. Lamb and Klaussner (1988) and Witkowski (1989) studied the response of *P. repens* to N and P applications. No leaf data were presented in these studies. Parvin (1986) reported on the use of plant and soil analysis in Hawaii

making fertiliser recommendations for *P. cynaroides*, *P. exima*, *P. neriifolia*, and hybrids 'Yellow Hebe' and 'May Day'. However, diagnostic standards developed overseas may need to be modified to suit local environmental conditions.

Pink Ice, which is a hybrid between *P. neriifolia* and *P. compacta*, was bred in Australia and has been widely planted since its release in 1981. To allow the development of a leaf-sampling procedure for Pink Ice, this paper presents data on variation in leaf nutrient composition with leaf position and sampling time. Data on growth and yield collected as part of this study will be published separately.

Materials and methods

Sites

The study was carried out on 3 commercial plantings in the Mount Lofty Ranges of South Australia. The climate is classified as Mediterranean, with cool, wet winters and dry, warm summers. Pest and disease control, irrigation, and fertiliser management were carried out by the grower. Annual rates of N and K applied were low, and no P was applied at any site. Plants at all sites were drip-irrigated. Plant ages were 5–7 years when sampling commenced in July 1990. Selected chemical and physical properties of the soil at each site are presented in Table 1.

Sampling procedure

To determine the magnitude of seasonal nutrient trends, leaf sampling was carried out monthly at each site from July 1990 to June 1993. At each sampling, at least 30 leaves were collected from healthy plants scattered throughout the planting. The index tissue sampled was a hardened, youngest fully expanded leaf (YFEL) from a shoot >40 cm long with a dormant vegetative terminal bud.

Sampling error

The reproducibility of the sampling procedure was tested by comparing the results of sampling uniform plantings at 2 sites on 5 consecutive occasions on the same day. A similar procedure was used by Cresswell (1989).

Variation in nutrient composition with leaf position along a shoot

To study the effect of sampling leaves next in age to the YFEL, the 3 consecutive leaves above (YFEL – 1, YFEL – 2, YFEL – 3) and below (YFEL + 1, YFE L + 2, YFEL + 3) were sampled. We collected leaves from 20 shoots on each of 6 plants. For each nodal position, leaves from all shoots on each plant were combined. This provided sufficient material for chemical analysis. The sampling was carried out at 2 commercial plantings in November 1990. November is the period of peak vegetative growth for Pink Ice (G. E. Barth unpublished data). George *et al.* (1989) reported that differences in nutrient composition with leaf nodal position were greater during peak vegetative flushing than midflush and completion of flushing.

Nutrient composition and removal by flowering stems

To estimate nutrient composition and removal by flowering stems, up to 8 flowering stems were collected from each site during the 1991 and 1992 harvests. For each sample, fresh and dry weights were determined before chemical analysis.

Table 1. Chemical and physical properties of the soil at each site

Phosphorus and potassium extracted in 1:100 soil:0.5 mmol $NaHCO_3$/L, 16 h shaking time (Colwell 1970)
Copper, zinc, manganese, and iron: DTPA extraction (Heanes 1981)

Depth (cm)	pH[A]	EC[A] (mS/cm)	Extractable nutrients (mg/kg) P	K	Cu	Zn	Mn	Fe	Organic C (%)[B]	Sand (%)	Silt (%)	Clay (%)
					Site 1							
0–15	4.8	0.06	15	113	0.7	1.7	3.9	44.5	2.7	85	10	4
15–30	5.2	0.03	16	95	0.3	0.5	0.7	42.6	0.9	79	12	10
30–40	5.3	0.02	17	83	0.2	0.5	0.5	41.9	0.8	77	11	12
40–70	5.4	0.02	5	60	0.2	0.3	0.2	22.7	0.4	76	10	13
					Site 2							
0–20	7.0	0.03	14	43	0.3	1.1	0.9	8.8	0.4	95	2	2
20–70	6.8	0.03	9	48	0.1	0.3	0.1	10.7	0.1	90	4	6
70–100	6.9	0.05	4	60	0.1	0.9	0.2	7.9	0.1	87	4	9
					Site 3							
0–15	6.2	0.03	64	92	0.7	2.0	1.7	45.0	1.4	98	1	1
15–40	6.0	0.03	13	37	0.3	0.4	0.3	35.3	0.2	96	2	2
40–80	6.2	0.06	2	83	0.1	0.8	0.1	14.0	0.3	38	2	60
80–100	6.2	0.08	4	73	0.1	0.4	0.1	11.5	0.2	54	4	42

[A] 1:5 soil:water. [B] Walkley and Black (1934).

Stem fractionation

To determine the nutrient composition of blooms (flowers), stem + leaves, and axillary shoots (young shoots arising from near the base of the bloom), a flowering stem was collected from each site in April 1992 and divided into these fractions for chemical analysis.

Soil sampling

To characterise the soil, samples were collected at each site in May 1992. Soil profiles were studied at up to 3 locations at each planting using a 7.5-cm auger. The depth of different soil horizons was determined by noting changes in soil texture or colour. Data are presented for a representative location at each site.

Analytical procedure

All plant samples were dried at 60–70°C in a forced-draught oven and ground to <1 mm before analysis. The samples were analysed for total N using a modified Kjeldahl method. Following selenious acid–sulfuric acid–hydrogen peroxide digestions, samples were analysed for K and P, by an autoanalyser procedure based on molybdate–vanadate method for P, and by flame photometry for K (Heanes 1981; Maier 1986). Calcium (Ca), magnesium (Mg), Na, sulfur (S), boron (B), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) were determined by inductively coupled plasma–atomic emission spectrometry following nitric acid digestion.

All soil samples were air-dried and ground to <2 mm before chemical analysis (Table 1).

Statistical methods

For each site, mean (± s.e.) monthly nutrient concentrations were determined for the 3 years of the study. The effect of leaf position on nutrient concentration was determined by analysis of variance. Correlation coefficients (r) for linear relationships between all nutrients in YFEL were determined. Only significant ($P<0.05$) r values are presented.

Results and discussion

Soil chemical and physical properties

Soil acidity varied from neutral throughout the profile at site 2 to highly acid at site 1 (Table 1). The mean (± s.e.) total number of stems harvested annually per plant over the 3 years was 63 ± 7 at site 1, compared with 39 ± 1 at site 2 and 57 ± 9 at site 3 (Barth *et al.* 1994). We suggest, therefore, that Pink Ice is tolerant of high soil acidity. Electrical conductivity (EC) values ranged from 0.02 to 0.08 mS/cm (Table 1), showing that soil salinity was low at all sites and not limiting yield.

We are not aware of any published critical soil P concentrations for proteas, even though many species may have a low tolerance of P (Cresswell 1991; Claassens 1981). An interesting feature in our study in relation to P nutrition is that extractable P concentrations in the 0–15 and 0–20 cm surface soils ranged from 64 mg/kg at site 3 to 14 and 15 mg/kg at sites 2 and 1, respectively (Table 1); however, this high residual P concentration was not associated with reduced yields. For example, mean total number of stems harvested annually per plant was 57 ± 9 at site 3, compared with 63 ± 7 at site 1, which was the highest yielding site.

The K requirement for optimum growth of proteas appears to be low; for example, Claassens (1981) recommended an application of up to 25 kg K/ha.year if extractable K values in the soil were very low (<20 mg/kg). Our values (43–113 mg/kg in the surface soils, Table 1) were high compared with this concentration.

Maier and Robinson (1986) classified soil fertility on the basis of percentage organic C: low (<1%), large response to applied N; moderate (1–2%), response to applied N uncertain; high (>2%), no response to applied N. Using this system, the soil at site 1 would be classified as highly fertile; site 2, infertile; and site 3, moderately fertile with regard to N. These data suggest that N fertiliser management should be different at the 3 sites.

Hannam (1985) presented data showing that for DTPA-extractable Cu, Zn, Mn, and Fe, respectively, deficient concentrations (mg/kg) were <0.2, <0.5, <1.0, and <2.5. Based on these interpretation standards, the concentrations of these micronutrients in the surface soils were not deficient (Table 1).

Sampling error

For N, P, K, Ca, Mg, Na, S, and B, the error associated with our sampling procedure and analytical error were acceptable, with coefficients of variation <15% (Table 2). Our estimates were less reliable for Cu, Zn, Mn, and Fe, with coefficients of variation up to 61.1% for Cu at site 2. Data reported by Cresswell (1989) for kiwifruit also showed that coefficients of variation for Cu, Zn, Mn, and P were higher than for N, K, Ca, and Mg. We suggest that to assess reliably Cu, Zn, Mn, and Fe status, a more intensive sampling of plants is required than was used in this study.

Table 2. Coefficients of variation (%) for leaf analysis data from two sites

	N	P	K	Ca	Mg	Na	S	B	Cu	Zn	Mn	Fe
Site 2	2.0	1.8	3.9	6.3	6.5	5.7	13.3	12.6	61.1	44.1	13.9	9.6
Site 3	2.4	4.8	1.6	6.2	9.1	12.2	5.4	7.1	14.1	31.6	31.7	24.2

Table 3. Effect of leaf position along a shoot on nutrient composition

Position relative to youngest fully expanded leaf (YFEL): minus, toward growing tip; plus, toward main stem

Leaf position	N	P	K	Ca	Mg	Na	S	B	Cu	Zn	Mn	Fe
				(%)						(mg/kg)		
YFEL – 3	0.84	0.13	0.39	0.91	0.08	0.25	0.13	15	8	28	66	45
YFEL – 2	1.05	0.09	0.53	0.38	0.11	0.21	0.12	10	11	n.a.	53	n.a.
YFEL – 1	1.05	0.09	0.52	0.39	0.11	0.22	0.12	10	10	53	49	52
YFEL	1.05	0.09	0.51	0.39	0.10	0.21	0.12	10	10	46	51	55
YFEL + 1	1.02	0.09	0.50	0.41	0.10	0.22	0.13	13	6	39	50	53
YFEL + 2	1.00	0.09	0.49	0.40	0.10	0.22	0.13	12	9	37	51	53
YFEL + 3	1.00	0.09	0.49	0.37	0.10	0.22	0.12	10	8	29	49	53
l.s.d. ($P = 0.05$)	0.04	0.02	0.02	0.05	0.01	0.02	n.s.	1	n.s.	9	8	6

n.a., not available; n.s., not significant.

Variation in nutrient composition with leaf position along a shoot

For both perennial and annual crops the effect of sampling leaves next in age to the index leaf (e.g. YFEL + 1, YFEL – 1 *v.* YFEL) on nutrient concentrations and, therefore, on interpretation can be significant, depending on the species and leaves studied (Bell *et al.* 1987; Clark and Gourley 1987; Cresswell 1989; George *et al.* 1989; Bell *et al.* 1990; Dole and Wilkins 1991; Lewis 1992). These studies show that greater emphasis should be given to studying the effect of leaf age or position when calibrating plant tests. We found that differences in nutrient composition of YFEL and other leaves up to YFEL – 2 and YFEL + 3 on vegetative shoots in November were of little practical significance (Table 3). Differences in concentrations between YFEL and YFEL – 3 were significant ($P<0.05$) for all nutrients except S and Cu. Sampling YFEL – 3 would therefore lead to errors in interpretation, but because it is obviously smaller than YFEL, it is easily identified.

Data reported by Parvin (1986) for *P. neriifolia* showed only minor differences in N, P, K, Mg, Ca, S, silicon, chloride, Cu, Fe, and Zn concentrations between 'juvenile' leaves (leaves expanded to almost full size but very soft and collected within the apical 5–8 cm of the stem) and 'mature' leaves (leaves fully expanded, firm, and collected from the basal portion of the most recent vegetative flush).

Nutrient composition and removal by flowering stems

The concentrations of nutrients were generally lower in flowering stems than YFEL (Table 4; Fig. 1). Nutrient removal by flowering stems, in order from greatest to least, was Ca > N > K > Na > S > Mg > P > Fe > Mn > Zn > B > Cu (Table 4). Such data are useful to formulate maintenance fertiliser strategies for Pink Ice. Claassens (1986) concluded that proteas do not remove large amounts of nutrients in harvested product. For example, nutrient removal (kg/ha) in flower heads of unfertilised *P. neriifolia* was reported to be in the order N (5.3) > Ca

Table 4. Mean (± s.e.) nutrient concentrations in, and nutrient removal by, flowering stems

	Concentration			Total nutrient removal		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
		Per cent			*(g/50 stems)*	
	0.42 ± 0.04	0.37 ± 0.02	0.37 ± 0.01	14.3 ± 1.9	14.8 ± 2.2	20.0 ± 5.6
P	0.03 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	1.2 ± 0.3	2.1 ± 0.3	2.7 ± 0.8
K	0.30 ± 0.02	0.18 ± 0.01	0.16 ± 0.01	10.4 ± 1.8	7.3 ± 1.0	9.1 ± 2.7
Ca	0.51 ± 0.05	0.59 ± 0.03	0.52 ± 0.03	18.5 ± 4.4	23.3 ± 2.9	28.1 ± 6.9
Mg	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	2.2 ± 0.5	2.5 ± 0.3	3.2 ± 0.6
Na	0.13 ± 0.01	0.19 ± 0.02	0.22 ± 0.02	4.2 ± 0.5)	7.2 ± 0.9	12.2 ± 3.3
S	0.06 ± 0.00	0.08 ± 0.01	0.06 ± 0.00	2.0 ± 0.4	2.9 ± 0.4	3.4 ± 0.9
		(mg/kg)			*(mg/50 stems)*	
B	9 ± 1	11 ± 0	12 ± 1	39 ± 8	49 ± 6	60 ± 13
Cu	3 ± 0	3 ± 0	3 ± 0	9 ± 2	14 ± 2	17 ± 3
Zn	14 ± 1	15 ± 1	19 ± 2	48 ± 8	58 ± 8	103 ± 2
Mn	43 ± 4	32 ± 2	42 ± 2	157 ± 35	127 ± 17	227 ± 58
Fe	51 ± 4	59 ± 5	30 ± 1	180 ± 35	246 ± 50	172 ± 50

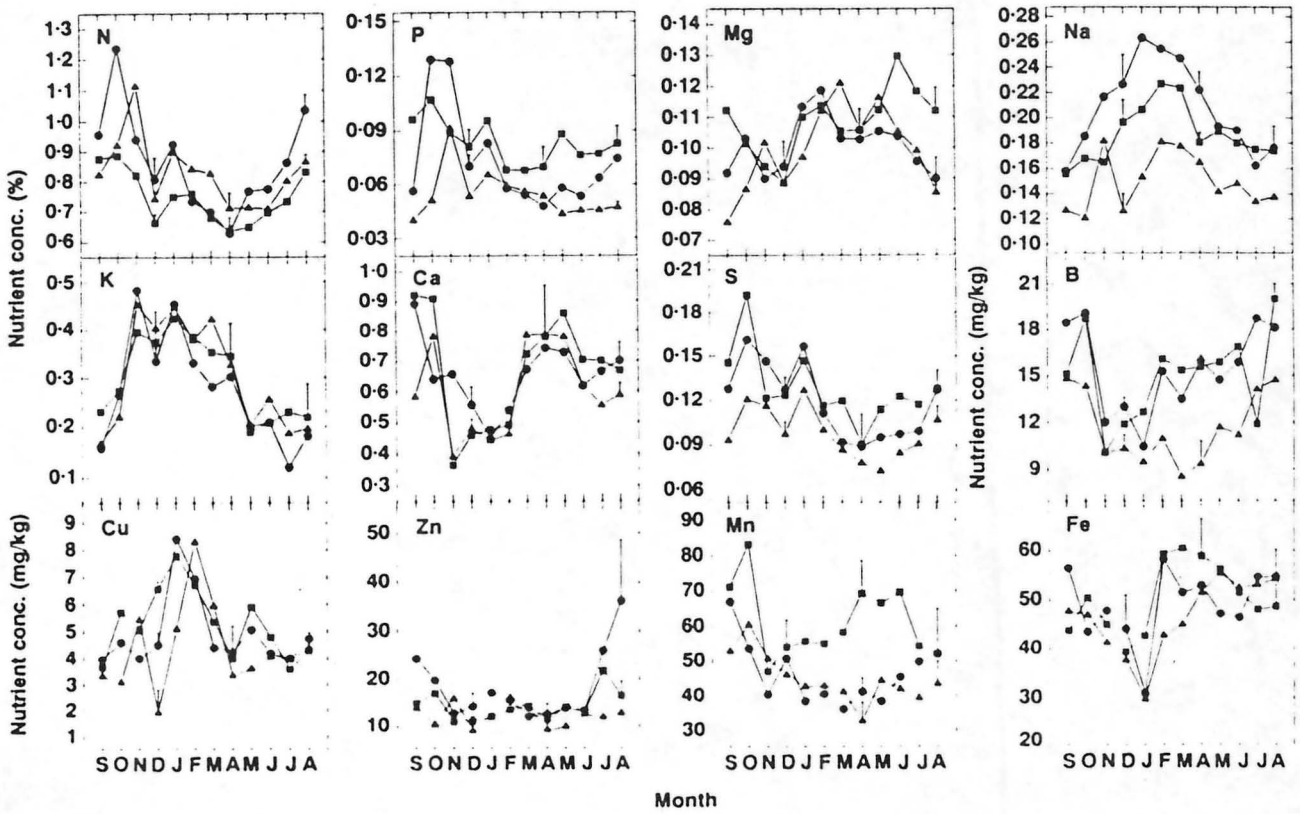

Figure 1. Seasonal changes in nutrient concentrations in youngest fully expanded leaves collected from *Protea* 'Pink Ice'. Data presented are mean concentrations over three years for site 1 (▲), site 2 (■), and site 3 (●). Vertical bars indicate representative standard errors.

and K (4) > P (0.8) > Mg (0.4). However, these estimates are low compared with our values based on flowering stems. For example, with 1850, 2900, and 1900 plants/ha at sites 1, 2 and 3, respectively, the amount (kg/ha) of nutrient removed was 26–43 N; 34–68 Ca; 17–21 K; 2–6 P; and 4–7 Mg.

Nutrient composition of stem fractions

Concentrations of N in axillary shoots and K in blooms were significantly higher than their concentrations in the other fractions (Table 5). Calcium and Mn concentrations in blooms were significantly lower than in axillary shoots and stem + leaf fractions.

Claassens (1986) reported the following nutrient concentrations (%) in blooms of *P. neriifolia*: N 0.53, P 0.08, K 0.4, Ca 0.4, and Mg 0.04. These concentrations are similar to our results (Table 5), except for Ca which was 135% higher in Claassens' study.

Seasonal variation

Seasonal trends in leaf nutrient composition have been reported for many perennial horticultural crops, and factors discussed to explain such trends include leaf age and position, retranslocation of nutrients, competition for nutrients between plant tissues, degree of nutrient stress (e.g. deficient *v.* adequate), growth dilution, vegetative flushing, fruit load, soil temperature, and nutrient mobility (Cresswell and Wickson 1986; George *et al.* 1989). Earlier studies (above) reported that mobile nutrients (e.g. N, P, K) usually show a decline in concentration in leaves with time, for example, from

Table 5. Mean (± s.e.) nutrient concentrations in different parts of flowering stems

	Stem + leaves	Axillary shoots	Bloom
	Per cent		
N	0.42 ± 0.01	0.59 ± 0.04	0.41 ± 0.01
P	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.00
K	0.22 ± 0.03	0.27 ± 0.04	0.48 ± 0.06
Ca	0.63 ± 0.12	0.61 ± 0.06	0.17 ± 0.03
Mg	0.06 ± 0.01	0.09 ± 0.01	0.05 ± 0.00
Na	0.21 ± 0.06	0.22 ± 0.04	0.22 ± 0.06
Cl	0.10 ± 0.02	0.08 ± 0.01	0.11 ± 0.01
S	0.05 ± 0.01	0.09 ± 0.01	0.08 ± 0.00
	(mg/kg)		
B	8 ± 2	11 ± 4	7 ± 1
Cu	2 ± 1	3 ± 1	4 ± 1
Zn	19 ± 2	16 ± 2	14 ± 1
Mn	43 ± 6	38 ± 3	11 ± 1
Fe	92 ± 12	96 ± 11	68 ± 37

spring to autumn. In contrast, nutrients of low or intermediate mobility (e.g. Ca, Mg, Mn, B, Fe) tend to show an increase with time. For many of the nutrients we monitored, the seasonal trends were consistent with those reported in these earlier studies.

Nitrogen. At sites 2 and 3, which were in the same growing district, N concentrations (Fig. 1) and stem elongation (G. E. Barth unpublished data) peaked in October. In contrast, at site 1, N concentrations and stem elongation peaked in November. The patterns of seasonal N concentrations therefore appear to be influenced by vegetative flushing. The seasonal decline in N concentrations may occur because N, which is a mobile element, is being retranslocated to new sinks such as blooms and axillary shoots during summer and autumn. Similar trends have been reported for total N concentrations in leaves of pecan (Cresswell and Wickson 1986), kiwifruit (Cresswell 1989), and custard apple (George *et al.* 1989), and for nitrate-N in grapes (Robinson and McCarthy 1985). Parvin (1986) also reported that N concentrations in leaves of *P. neriifolia* were highest in spring (1.37%) and lowest in winter (0.78%).

Maximum N concentrations ranged from 1.2% at site 3, which was classified as moderately fertile, to 0.6% at site 2, which was classified as infertile with regard to soil N. Yield of flowering stems and stem elongation were significantly lower at site 2 than sites 1 and 3 (G. E. Barth unpublished data). The lower stem yields and leaf N concentrations at site 2 suggest that N management may have been inadequate at this site.

Published diagnostic standards for N in *Protea* species are limited. For N concentrations in youngest mature blades (YMB) of *P. cynaroides* and *P. repens* sampled in August, Price (1986) reported adequate ranges of 1.2–1.3 and 1.4–1.6%, respectively. These values are high compared with the range in N concentrations of YFEL sampled in August in our study (Fig. 1). For *P. neriifolia,* the seasonal N concentrations reported by Parvin (1986) are similar to those found in our study for Pink Ice.

Phosphorus. Seasonal trends in P concentrations were similar to N (Fig. 1). Parvin (1986) also reported that P concentrations were highest in spring and summer (0.10%) and lowest in winter (0.06%). Cresswell (1991) suggested the following standards (% P) for the interpretation of the P status of Pink Ice, using recently matured leaves: low–deficient <0.06, desirable 0.06–0.27, high 0.27–0.57, toxic >0.57. Our data for site 1 (the highest yielding site) suggests that, depending on sampling time, P concentrations of 0.05–0.13% are adequate for high yield and quality. We therefore suggest that the adequate P concentration range in YFEL is lower than suggested by Cresswell based on greenhouse studies. For *P. cynaroides* and *P. repens*, Price (1986) reported adequate P concentration ranges in YMB sampled in August of 0.04–0.06 and 0.09–0.10%, respectively. Phosphorus concentrations in YFEL of Pink Ice sampled in August (Fig. 1) are consistent with the data for *P. cynaroides.*

Potassium. At all sites K concentrations increased dramatically during the spring vegetative flush and declined in January–May (Fig. 1). The fall in leaf K concentrations may be related to bloom development during this period. Most blooms were harvested in March–June (G. E. Barth unpublished data), and because blooms have a higher K concentration than stem + leaf and axillary shoot fractions (Table 5), they are an important sink for K. Reduced mineralisation with lower soil temperatures may also contribute to this decline (George *et al.* 1989). A similar seasonal trend in K concentrations was reported by Parvin (1986). In our study, the K concentrations in YFEL sampled in August ranged from 0.18 to 0.22%. These values are low compared with the adequate ranges presented for *P. cynaroides* (0.34–0.73%) and *P. repens* (0.31–0.52%) by Price (1986) and for *P. neriifolia* (0.52%) by Parvin (1986).

Calcium. The seasonal trends in Ca, which is an immobile element, may reflect leaf age (Fig. 1). Leaves sampled in March–May were older than leaves sampled during October–December, since vegetative flushing occurs during spring and early summer, and Ca continues to accumulate as leaves age (Stephenson *et al.* 1986). Price (1986) presented adequate Ca concentration ranges of 0.52–1.00 and 0.45–0.73% for YMB of *P. cynaroides* and *P. repens,* respectively, sampled in August. Our data for Pink Ice are consistent with these ranges. Parvin (1986) showed that Ca concentrations of *P. neriifolia* were lower in chlorotic leaves (0.27–0.39%) than green leaves (0.40–0.84%).

Magnesium. Seasonal trends in Mg concentrations were not consistent between sites (Fig. 1). During spring and summer, Mg concentrations at sites 1 and 3 increased. Concentrations during winter were higher at site 2 than sites 1 and 3, and at site 2, concentrations decreased during winter–spring. Parvin (1986) reported that Mg concentrations in *P. neriifolia* were highest during winter (0.24%) and spring (0.24%) and decreased during summer (0.13%) and autumn (0.06%). Those concentrations in winter and spring were much higher than our values for Pink Ice, and the seasonal trend was not consistent with our data (Fig. 1). Price (1986) reported adequate Mg concentration ranges for *P. cynaroides* and *P. repens* during August (0.10–0.12 and 0.06–0.16%, respectively). Our data for Pink Ice suggest that Mg concentrations as low as 0.085% in YFEL sampled in August are adequate.

Sodium. Although Na concentrations in YFEL varied between sites, seasonal trends were consistent and

concentrations peaked during January–February (Fig. 1). The variation in Na concentrations between sites may be due to differences in the amount and quality of irrigation water applied. The Na concentrations in YFEL found in our study fall within the adequate range (0.13–0.21%) for Na in YMB of *P. cynaroides* sampled in August (Price 1986). In contrast, they are high compared with the adequate range presented for *P. repens* (0.04–0.07%).

Sulfur. The seasonal trends for S were similar to those for N and P (Fig. 1). Parvin (1986) reported S concentrations in green, healthy leaves of *P. neriifolia* of 0.08–0.11%. Adequate concentration ranges of 0.18–0.24 and 0.24–0.29% S in YMB sampled in August were presented by Price (1986) for *P. cynaroides* and *P. repens*, respectively. These values are high compared with our data for Pink Ice and Parvin's data for *P. neriifolia*.

Boron. The concentration of B in leaves increased during January–August (Fig. 1). Since B is an immobile element, this trend may be due to increasing leaf age. The B concentrations in YFEL sampled in August are consistent with the adequate ranges cited by Price (1986) for *P. cynaroides* (14–24 mg/kg) and *P. repens* (19–24 mg/kg).

Copper. Concentrations in YFEL peaked during January–February (Fig. 1) in the range 7–8 mg/kg, consistent with the range 6–8 mg/kg reported by Parvin (1986) for *P. neriifolia*. Concentrations in YFEL sampled in August were 4–5 mg/kg. These values are higher than, or at the upper end of, the adequate ranges reported for *P. cynaroides* (2–3 mg/kg) and *P. repens* (4 mg/kg) (Price 1986).

Zinc. There were no consistent trends between sites in the seasonal patterns of Zn concentrations in the YFEL (Fig. 1). At sites 2 and 3, concentrations in YFEL peaked in July–August. Based on data for site 1 (Barth *et al.* 1994), we suggest that concentrations of 10–15 mg Zn/kg in YFEL throughout the year are adequate. This range is lower than the adequate ranges reported for *P. cynaroides* (19–27 mg/kg) and *P. repens* (26–35 mg/kg) (Price 1986).

Manganese. Concentrations of Mn in the YFEL peaked during September–October, and the trends in concentrations during December–June were not consistent between site 2 and sites 1 and 3 (Fig. 1). Concentrations in leaves of Pink Ice in our study, and those reported by Parvin (1986) in green (49–89 mg/kg) and chlorotic (31–70 mg/kg) leaves of *P. neriifolia*, were much lower than the adequate ranges presented by Price (1986) for *P. cynaroides* (145–265 mg/kg) and *P. repens* (208–220 mg/kg). We suggest for sampling during July–August, Mn concentrations of 40–50 mg/kg in YFEL of Pink Ice are adequate.

Iron. Iron concentrations in YFEL were lowest during the spring and early summer vegetative flush (Fig. 1). This seasonal pattern was similar to that of other immobile nutrients such as Ca and B. Concentrations in YFEL sampled in August (Fig. 1) were similar to the adequate range (38–51 mg/kg) in YMB of *P. cynaroides* (Price 1986).

Table 6. Correlation coefficients (r) between nutrients
Relationships presented are significant at $P = 0.05$

	N	P	K	Ca	Mg	Na	B
P	0.47						
K		0.29					
Ca			–0.62				
Na		0.40	0.27		0.51		
S	0.36	0.53	0.40	–0.50			
B			–0.45				
Cu			0.44		0.50	0.60	
Zn	0.58						0.41
Mn	–0.31						0.67
Fe			–0.53				0.43

Correlations between nutrients

Significant ($P<0.05$) positive and negative correlations were found between some nutrients (Table 6). These correlations may indicate interactions which can affect critical concentrations, induce deficiencies or toxicities, and modify growth response, depending on nutrient supply (Lewis *et al.* 1993). Therefore, to ensure correct interpretation when diagnosing nutrient disorders, interactions between nutrients must be considered.

Sampling for leaf analysis

Many plant parts, ranging from whole shoots to key index tissues (e.g. whole leaves, petioles, blades), have been used to calibrate plant tests for annual and perennial species. For reliable interpretation the plant part chosen should be sensitive to variations in nutrient supply; show a sharp transition between deficiency and

Table 7. Range in mean nutrient concentrations for the highest yielding sites (sites 1 and 3) at two sampling times

Nutrient	December–February	May–August
	Per cent	
N	0.82–0.83	0.77–0.86
P	0.06–0.07	0.05–0.06
K	0.37–0.41	0.18–0.21
Ca	0.46–0.51	0.63–0.68
Na	—	0.14–0.18
S	0.11–0.13	0.09–0.10
	(mg/kg)	
Cu	—	3.5–4.5
Zn	12–15	—
Mn	43–44	—
Fe	—	51–54

dequacy; be easily identifiable, to minimise sampling error; have stable nutrient concentrations, and therefore critical values or ranges, over time; and have a yield response that is highly correlated with nutrient concentration at the time of sampling (Stephenson and Cull 1986; Lewis *et al.* 1993). In contrast with annual species, diagnostic standards for perennial crops are usually established by monitoring the nutrient status of highly productive plantings over a number of years. Of the factors listed above, nutrient stability has been the main basis for determining the desirable sampling time for perennial species. The preferred sampling time is when seasonal variation in leaf composition is smallest. However, Cresswell and Wickson (1986) and George *et al.* (1989) report that a single sampling time suitable for all nutrients is often not available because of [illegible]rences in their seasonal trends.

Inspection of the seasonal trends presented in Figure 1 shows that concentrations of N, P, K, Ca, Na, S, Cu, and Fe were fairly stable over May–August. Similarly, concentrations of N, P, K, Ca, S, Zn, and Mn were relatively stable during December–February, after completion of the spring vegetative flush. However, the supply of hardened YFEL during December may be limited; therefore, sampling would be preferred during January and February. For these sampling periods, nutrient concentrations associated with high yields are presented in Table 7. These ranges may be considered as tentative adequate or desirable nutrient concentrations to interpret plant test data. However, to establish a reliable plant test for Pink Ice based on sampling at these times, further experimental work is required to determine the relationships between nutrient concentration and yield response.

We are not aware of any studies published on the sensitivity of nutrient concentrations in YFEL to v[illegible]ions in nutrient supply. N. A. Maier (unpublished data) found that increasing the annual rate of applied N from 0 to 50 g/plant significantly ($P<0.05$) increased N concentrations in YFEL of Pink Ice sampled in January, from 0.91 to 1.24%. These data show that N concentrations in YFEL are sensitive to N supply.

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References

Bell, R. W., Brady, D., Plaskett, D., and Loneragan, J. F. (1987). Diagnosis of potassium deficiency in soybean. *Journal of Plant Nutrition* **10**, 1947–53.

Bell, R. W., Kirk, G., Plaskett, D., and Loneragan, J. F. (1990). Diagnosis of zinc deficiency in peanut (*Arachis hypogaea* L.) by plant analysis. *Communications in Soil Science and Plant Analysis* **21**, 273–85.

Claassens, A. S. (1981). Soil preparation and fertilisation of proteas. Flowers and Ornamental Shrubs B.14/1981. Department of Agriculture and Fisheries, Pretoria, South Africa.

Claassens, A. S. (1986). Some aspects of the nutrition of proteas. *Acta Horticulturae* **185**, 171–9.

Clark, R. B., and Gourley, L. M. (1987). Leaf position and genotype differences for mineral element concentrations in sorghum grown on tropical acid soil. *Journal of Plant Nutrition* **10**, 921–35.

Colwell, J. D. (1970). A statistical–chemical characterisation of four great soil groups in southern New South Wales, based on orthogonal polynomials. *Australian Journal of Soil Research* **20**, 221–38.

Cresswell, G. C. (1989). Development of a leaf sampling technique and leaf standards for kiwifruit in New South Wales. *Australian Journal of Experimental Agriculture* **29**, 411–17.

Cresswell, G. C. (1991). Assessing the phosphorus status of proteas using plant analysis. *In* '6th Biennial International Protea Association Conference, Perth, September 1991'.

Cresswell, G. C., and Wickson, R. J. (1986). Seasonal variation in the nutrient composition of the foliage of pecan (*Carya illinoensis*). *Australian Journal of Experimental Agriculture* **26**, 393–7.

Dole, J. M., and Wilkins, H. F. (1991). Relationships between nodal position and plant age on the nutrient composition of vegetative poinsettia leaves. *Journal of American Society of Horticultural Science* **116**, 248–52.

George, A. P., Nissen, R. J., and Carseldine, M. L. (1989). Effect of season (vegetative flushing) and leaf position on the leaf nutrient composition of *Annona* spp. hybrid cv. Pink's Mammoth in south-eastern Queensland. *Australian Journal of Experimental Agriculture* **29**, 587–95.

Graley, A. M. (1982). Nutrient status of apple trees in two productive orchards in Tasmania. *Australian Journal of Experimental Agriculture and Animal Husbandry* **22**, 232–8.

Hannam, R. J. (1985). Micronutrient soil test. *In* 'Proceedings of the Soil and Plant Analysis Training Course 1984/85'. (Ed. D. J. Reuter.) (South Australian Department of Agriculture.)

Heanes, D. L. (1981). Laboratory methods of soil and plant analysis. Department of Agriculture, South Australia.

Lamb, A. J., and Klaussner, E. (1988). Response of the fynbos shrubs *Protea repens* and *Erica plukenetii* to low levels of nitrogen and phosphorus applications. *South African Journal of Botany* **54**, 588–64.

Leece, D. R. (1976). Diagnosis of nutritional disorders of fruit trees by leaf and soil analyses and biochemical indices. *Journal of the Australian Institute of Agricultural Science* **42**, 3–19.

Lewis, D. L. (1992). Effect of plant age on the critical inorganic and total phosphorus concentrations in selected tissues of subterranean clover (cv. Trikkala). *Australian Journal of Agricultural Research* **43**, 215–23.

Lewis, D. C., Grant, I. L., and Maier, N. A. (1993). Factors affecting the interpretation and adoption of plant analysis services. *Australian Journal of Experimental Agriculture* **33**, 1053–66.

Maier, N. A. (1986). Potassium nutrition of irrigated potatoes in South Australia. 2. Effect on chemical composition and the prediction of tuber yield response by plant analysis. *Australian Journal of Experimental Agriculture* **26**, 727–36.

Maier, N. A., and Robinson, J. B. (1986). Soil analysis for field grown vegetables in SA. Factsheet FS8.83, South Australian Department of Agriculture.

Parvin, P. E. (1986). Use of tissue and soil samples to establish nutritional standards in protea. *Acta Horticulturae* **185**, 145–53.

Price, G. H. (1986). Ornamentals. *In* 'Plant Analysis: an Interpretation Manual'. 1st Edn. (Eds D. J. Reuter and J. B. Robinson.) pp. 188–217. (Inkata Press: Melbourne, Sydney.)

Reuter, D. J., and Robinson, J. B. (1986). 'Plant Analysis: an Interpretation Manual'. 1st Edn. (Inkata Press: Melbourne, Sydney.)

Robinson, J. B., and McCarthy, M. G. (1985). Use of petiole analysis for assessment of vineyard nutrient status in the Barossa district of South Australia. *Australian Journal of Experimental Agriculture* **25**, 231–40.

Stephenson, R. A., and Cull, B. W. (1986). Standard leaf nutrient levels for bearing macadamia trees in south east Queensland. *Scientia Horticulturae* **30**, 73–82.

Stephenson, R. A., Cull, B. W., Mayer, D. G., Price, G., and Stock, J. (1986). Seasonal patterns of macadamia leaf nutrient levels in south east Queensland. *Scientia Horticulturae* **30**, 63–71.

Walkley, A., and Black, T. A. (1934). An examination of the Degtjariff method of determining soil organic matter and a proposed modification of the chromic acid filtration method. *Soil Science* **37**, 29.

Witkowski, E. T. F. (1989). Effects of nutrients on the distribution of dry mass, nitrogen and phosphorus in seedlings of *Protea repens* (L.) L. (Proteaceae). *New Phytologist* **112**, 481–7.

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Control of experimental *Phytophthora cinnamomi* stem infections of *Rhododendron*, *Leucadendron* and *Eucalyptus* by dimethomorph, fosetyl-Al and metalaxyl

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Summary. The efficacy of dimethomorph, (*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-acryloyl] morpholine, in controlling *Phytophthora cinnamomi* stem infections of *Rhododendron*, *Leucadendron* and *Eucalyptus* was compared with that of fosetyl-Al, metalaxyl and phosphonate (potassium dihydrogen phosphonate) in a phytotron and greenhouse. The plants were inoculated on the stem and the effect of the fungicides applied either as foliar sprays or root drenches on lesion development was measured.

The results showed that dimethomorph inhibited lesion extension when applied as a soil drench at rates of 0.6 and 1.2 mg a.i./mL. When used as a soil drench dimethomorph was about as effective as fosetyl-Al and somewhat less effective than metalaxyl. When applied as foliar sprays, dimethomorph was ineffective and phosphonate was markedly superior to fosetyl-Al. Single applications of the fungicides tested were not able to kill *P. cinnamomi* in established infections within the duration of the experiment and under the test conditions which strongly favoured the fungus. Dimethomorph was slightly phytotoxic to *Eucalyptus sieberi* at dosages of 1.2 mg a.i./mL.

Introduction

Phytophthora cinnamomi Rands can produce lethal infections on major roots and root collars of many plant species and often causes considerable losses in forestry and agriculture. Within the last 15 years, several systemic fungicides that are active against species of *Phytophthora* have been developed but the list is small when compared to those that are active on the plant surface (British Crop Protection Council 1983). Systemic fungicides offer superior protection against *P. cinnamomi* root infection in comparison to the prophylactic types. With the possibility of a build up of fungal resistance, there is a need for a constant search for new systemic fungicides active against *Phytophthora* species (Schwinn 1983).

Recently, Shell International Chemical Company Pty Ltd produced a chemical that is active against many pathogens belonging to the Peronosporaceae. It has a proposed name, 'dimethomorph', with the chemical name (*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl] morpholine (IUPAC). Preliminary test data supplied by the Shell Company state that it has both curative and protective functions and can be translocated upwards when applied to roots.

The objective of these tests was to examine the activity, *in vivo*, of dimethomorph against *P. cinnamomi* and compare its efficacy with fosetyl-Al, metalaxyl and phosphonate in containing stem infections of *P. cinnamomi* in 3 species of plants.

Materials and methods

Plants were grown for at least 12 weeks, prior to testing, either in a phytotron (27°C day and 19°C night temperatures, 12 h day length and a light intensity of approximately 12 E/m^2.s at a distance of 360 mm above the top-most shoots) or in a greenhouse that was maintained at 22 ± 2°C without supplementary lighting.

All plants were fertilised with a slow-release, commercial fertiliser, except for *Leucadendron* species, where a slow-release, low phosphorus fertiliser was used. The plants were carefully maintained and were disease-free when the tests commenced.

Source of inoculum and fungicide application

The inoculum of *P. cinnamomi* (No. MS 10) used in all tests was isolated from the stem of a diseased native *Eucalyptus sieberi* Sm. tree growing in the Mullungdung State Forest 230 km south-east of Melbourne. Inoculations were made by inserting a small piece from the advancing edge of a 7-day-old culture of *P. cinnamomi*, grown on V-8 juice agar at room temperature, into an incision 2–3 mm long in the outer cortex of the test plant and covering it with a thick layer of vaseline. The fungicides were applied either as soil drenches or foliar sprays 1–5 days prior to inoculating the stems. The effectiveness of the fungicides was tested by measuring the size of the lesions and making re-isolations of *P. cinnamomi* on antibiotic agar (Eckert and Tsao 1962).

In all tests, a corresponding number of uninoculated plants was treated with fungicides and compared with uninoculated, untreated controls to determine whether the fungicides were phytotoxic at the dosages used.

Experimental design and measurements

Experiment 1. The objective of this experiment was a comparison of the efficacy of foliar applications of dimethomorph, fosetyl-Al and phosphonate in controlling stem infections on a *Rhododendron ponticum* hybrid (G. W. Leak 2426).

This experiment was carried out in the phytotron. The 12-month-old plants were supplied by a commercial grower in 500 mL pots containing a mixture of peat and decomposed bark. Because of some uncertainty regarding the pesticides applied by the grower, these plants were repotted into 1 L pots and grown for a further 4 months prior to starting the tests.

Dimethomorph was used at concentrations of 0.3, 0.6 and 1.2 mg a.i./mL and both fosetyl-Al and phosphonate were used at a concentration of 1 mg/mL molar equivalent of PO_3^{3-}. Only 1 fungicidal application was used and the stems were inoculated 3 days after the fungicides were applied. All plants were sprayed to run-off and the soils protected from spray drift by paper towels. The inoculation points on the green stem were shielded with adhesive tape prior to applying the fungicidal sprays. The amount of disease was assessed 31 days after the stems were inoculated. There were 7 replicates in each treatment. Re-isolation tests on antibiotic agar were carried out 31 days after inoculation, from the inoculation point, the apical bud and the lowest visible limit of lesion extension on the stem below the inoculation point.

Experiment 2. The objective of this experiment was a comparison of the efficacy of dimethomorph with fosetyl-Al and metalaxyl in controlling stem infections of *P. cinnamomi* when the fungicides were applied as a root drench. This test was carried out in the phytotron. Dimethomorph was used at concentrations of 0.6 and 1.2 mg a.i./mL, fosetyl-Al at a concentration of 1 mg a.i./mL molar equivalent of PO_3^{3-} and metalaxyl at a concentration of 2 mg a.i./mL. There were 6 replications in each treatment, each pot contained a single plant and 50 mL of fungicide was applied to the soil in each pot, so that each plant received either 0.03 or 0.06 mg a.i./mL of dimethomorph or 0.005 mg a.i./mL of phosphonate.

The rhododendrons were inoculated 4 days after applying the root drench and the results assessed 72 days after inoculation. Re-inoculations for *P. cinnamomi* were made above, at the point of inoculation, and below the visible external lesion.

Experiment 3. The objective of this test and the conditions under which it was conducted were similar to those of experiment 2. However, a disease sensitive eucalypt, *Eucalyptus sieberi*, was used in place of the *Rhododendron*. A single seedling of *E. sieberi* was raised in 2 L capacity pots containing a krasnozem from a mixed eucalypt species forest at Mount Dandenong. The test was carried out in the phytotron and a single concentration of dimethomorph (0.6 mg a.i./mL) was used.

Experiment 4. This experiment was also similar to experiments 2 and 3 but was carried out in a greenhouse and *Leucadendron salignum* x *L. laureolum* (Silvan Red) was used as the test plant. The plants were raised singly in 1 L capacity pots in a mixture of peat and decomposed bark and had been sprayed to run-off point with a solution containing 1 mg a.i./mL of Foli-R-Fos (potassium dihydrogen phosphonate) by a commercial grower. Because of this treatment, the plants were held for 6 months prior to testing. The plants were about 30 months old when tested and there were 6 replicates per treatment.

The fungicide concentrations were the same as those used in experiment 3 but dimethomorph drench was compared with metalaxyl drench and phosphonate spray. Two branches of each plant were inoculated 5 days after application of the fungicides. The plants were assessed for disease symptoms 30 and 65 days after spraying. Re-isolations for *P. cinnamomi* were attempted at the inoculation point and advancing edge of the lesions 65 days after spraying.

Experiment 5. The objective of this experiment was the determination of the dosage levels of dimethomorph (applied as a single soil drench) required to contain *P. cinnamomi* stem infections on 8-month-old *E. sieberi* seedlings. Metalaxyl was used as a comparative standard. The treatment applied to each pot was either 50 mL of concentrations 0.05, 0.15, 0.3, 0.6 or 1.2 mg a.i./mL of dimethomorph, or 2 mg a.i./mL of metalaxyl. All treatments were inoculated 5 days after the fungicides were applied and the results assessed after 30 days. Assessments were made of seedling deaths and stem girdling, lesion length, containment of infection, direction in which the lesion developed, and phytotoxicity. There were 20 replicates in each treatment.

Statistical analyses. All data were analysed using analysis of variance.

Results

Experiment 1

All *Rhododendron* plants were growing rapidly when the experimental period started. Seven days after inoculation, black, necrotic tissue surrounded by a longer brick-red coloured zone formed at inoculation point. The incisions produced on uninoculated controls healed rapidly without discolouration. In some inoculated plants, the apical bud and young leaves turned red and wilted producing crown rot.

Re-isolations showed that *P. cinnamomi* could be recovered from all inoculation points, the brick-red

Table 1. Effect of fungicide treatments (n = 7) (applied as a single foliar application) on lesion size in stems of *Rhododendron* after inoculation with *P. cinnamomi*, and the number of re-isolations of *P. cinnamomi* from the apical bud, inoculation point and below the stem lesion

Means in columns followed by different letters are significantly different at P=0.05

Treatments (mg a.i./mL)	Mean lesion size (cm)		No. of plants with crown rot	No. re-isolations of *P. cinnamomi* from:		
	Red zone	Black zone		Bud	Inoc. point	Below lesion
Dimethomorph, 0.3	116.5b	16.7b	7	5	7	5
Dimethomorph, 0.6	109.8b	16.2b	7	5	7	5
Dimethomorph, 1.2	116.8b	15.6b	6	4	7	6
Fosetyl-Al, 1.0	97.9b	10.9b	6	n.t.	n.t.	n.t.
Phosphonate, 1.0	32.8a	8.9a	0	n.t.	n.t.	n.t.
Control						
Not inoculated	0	0	0	0	0	0
Inoculated	90.4b	18.4b	6	3	6	6

n.t., not tested.

coloured zone and the discoloured apical bud (Table 1).

The effects of a single foliar application of fungicides on lesion development and crown rot are shown in Table 1. Phosphonate (1 mg a.i./mL) sprayed on the leaves produced a significant (P<0.01) reduction in lesion size and there was no evidence of crown rot. The fungus was re-isolated from the lesions and diseased stem apices showing that dimethomorph and the single concentration of fosetyl-Al had no significant influence on lesion development.

Applications of phosphonate and fosetyl-Al produced a zone of green-coloured tissue around the black, necrotic lesion which was, in turn, surrounded by the brick-red discoloured zone. These green-islands were somewhat similar to the 'green-islands' produced by some foliar rust fungi.

Table 2. Comparison of the efficacy of dimethomorph root drench with metalaxyl and fosetyl-Al root drenches in controlling lesions (n=6) developed after inoculating *Rhododendron* with *Phytophthora cinnamomi*, and number of re-isolations of *P. cinnamomi* from top, inoculation point and bottom of stem lesion

Means with different letters are significantly different (P<0.05)

Treatments (mg a.i./mL)	Mean lesion length	No. re-isolations of *P. cinnammoni* from lesions		
		Top	Inoc. point	Bottom
Dimethomorph, 0.6	52.8a	6	6	4
Dimethomorph, 1.2	70.4b	4	6	2
Fosetyl, 1.0	48.8a	3	6	2
Metalaxyl, 1.0	51.1a	1	6	4
Control				
Inoculated	70.0b	4	6	4
Not inoculated	0	0	0	0
Wound only	0	0	0	0

Experiment 2

By the time this test commenced, the flush of new growth on the rhododendrons had matured. The lesions were generally smaller in the untreated controls (cf. Tables 1 and 2), possibly due to increased tissue maturation, and no crown rot developed. The results (Table 2) showed that when dimethomorph was used as a root drench at a concentration of 0.6 mg a.i./mL there was a significant (P<0.05) reduction in lesion extension when compared with the untreated, inoculated controls, and it was as effective as fosetyl-Al and metalaxyl in reducing lesion size. However, the higher concentration of dimethomorph did not significantly reduce lesion development. Re-isolation showed that a single application of these fungicides could not kill *P. cinnamomi* within an established lesion under the experimental conditions.

Experiment 3

Eucalyptus sieberi is susceptible to *P. cinnamomi* root and stem infection and lesion development is easy to monitor (Marks *et al.* 1981). The results (Table 3) showed that all 3 fungicides produced a significant (P<0.01) reduction in lesion extension when applied as a root drench. Lesion area in the treated plants was reduced by more than two-thirds when compared with the untreated, inoculated controls. Once again single applications of the root drenches were not able to eliminate *P. cinnamomi* from an established infection under these experimental conditions.

Experiment 4

Leucadendron (Silvan Red) is extremely susceptible to *P. cinnamomi* stem infection, and the conditions in the greenhouse (even temperatures between 20 and 24°C) were conducive to very rapid disease development. The

Table 3. Comparison of efficacy of dimethomorph root drench with root drenches of fosetyl-Al and metalaxyl in controlling *Phytophthora cinnamomi* stem infection of *Eucalyptus sieberi* and number (n=6) of re-isolations from the top, inoculation point and below the lesion

Means with different letters are significantly different (P<0.01)

Treatments (mg a.i./mL)	Lesion measurements Length (cm)	Area (cm^2)	No. of re-isolations from lesions Top	Inoc. point	Bottom
Dimethomorph, 0.6	6.7a	3.21	6	6	6
Metalaxyl, 2.0	5.3b	3.75	6	6	6
Fosetyl-Al, 1.0	7.6a	5.00	6	6	6
Control	18.0b	16.10	6	6	6

Table 4. Comparison of the efficacy of dimethomorph and metalaxyl root drenches (n=6) with phosphonate foliar spray in controlling the spread of *P. cinnamomi* in *Lencadendron* 'Silver Red' and number of positive recoveries of *P. cinnamomi* out of 36 attempts for each treatment

Treatments (mg a.i./mL)	Disease symptoms after: 30 days Girdled	30 days Dead	65 days Girdled	65 days Dead	No. of re-isolations
Control	6	0	6	6	36
Dimethomorph, 0.6	2	0	3	3	32
Metalaxyl, 2.0	1	0	1	1	18[A]
Phosphonate, 1.0	3	0	4	4	30[B]

[A] *P. cinnamomi* was isolated from all inoculation points.
[B] One replicate gave negative results, probably because the inoculum did not take.

results (Table 4) showed that the inoculated, untreated controls were rapidly girdled and the lesions extended quickly into the inoculated shoots and lower foliage. However, plants did not die immediately and only started to wither 45 days after being girdled. Under these greenhouse conditions, neither root drenching with dimethomorph or metalaxyl nor a single foliar spray of phosphonate provided complete protection, although there was some reduction in mortality when compared with the untreated controls. Although none of the fungicides was able to kill *P. cinnamomi* within an established infection, there were fewer re-isolations from Silvan Reds treated with metalaxyl. There was no evidence of any fungicidal phytotoxicity.

Experiment 5

When 50 mL of dimethomorph was applied at concentrations of 1.2 mg a.i./mL to the soil in each pot, 2 out of 20 seedlings began wilting within 48 h and eventually died. However, there was no evidence of phytotoxic shock in the other 18 seedlings. Both metalaxyl (2 mg a.i./mL) and dimethomorph applied at the rate of 1.2 mg a.i./mL produced a significant (P<0.01) reduction in lesion length and downward movement of infection and increased the amount of wound periderm (necrophylactic periderm) formed around parts of the lesions (Table 5). Seedling mortality and stem girdling decreased between dosage rates of 0.15 and 0.6 mg a.i./mL dimethomorph. However, these trends were not statistically significant when compared with the controls.

There was some evidence in all these tests that the highest dosage rate of 1.2 mg a.i./mL dimethomorph was slightly phytotoxic. It produced a mild, but transient, chlorosis in the *Rhododendron* and some damage (noted already) in young eucalypt seedlings. It was not toxic to Silvan Red or older *E. sieberi* seedlings.

Discussion

Shephard (1987) stressed the importance of ascertaining *inter alia*, the mobility of systemic fungicides with prophylactic effects. The stem inoculation technique used in these tests was designed to compare the systemic activity of the various fungicides

Table 5. Comparison of various dosages of dimethomorph applied as a soil drench with metalaxyl in controlling stem lesions (n=20) of *Phytophthora cinnamoni* on *Eucalyptus sieberi* and number of symptoms indicating active infection or recovery from infection

Means with different letters are significantly different (P<0.01)

Treatment (mg a.i./mL)	Mean lesion length (mm)	No. dead or stem girdled	No. of spreading lesions	No. of infected root collars	No. showing occlusions
Control	138a	3	13	8	7
Metalaxyl, 2.0	34b	1	1	0	19
Dimethomorph					
1.2	75b	4	2	0	13
0.6	121a	10	9	0	10
0.3	144a	12	13	0	7
0.15	161a	11	9	0	8
0.05	166a	13	5	0	15

being tested after they had been either applied to soil or sprayed on foliage. It provided a measure of the ability of the plant to absorb and translocate the fungicide and provided visible evidence of the interaction between host, pathogen and fungicide, such as appearance of the 'green islands' surrounding the inoculation points in the green stems of the *Rhododendrons* and the reduction in lesion lengths as shoots matured (Tables 1 and 2). In many cases, necrophylactic periderm formed as the infection was contained (Table 5). By isolating the pathogen from various tissues it is also possible to test the 'curative' effect of the fungicide and assess the risk of disease re-appearing after the effects of the fungicide have been reduced.

The results of these *in vivo* tests showed that dimethomorph was not effective, as a foliar spray, in controlling stem infection produced by *P. cinnamomi*. However, at a dosage rate of either 0.6 or 1.2 g a.i./mL it could be absorbed through the roots and transported to the above-ground parts of 3 unrelated genera of plants where it restricted *P. cinnamomi* lesion development. Dimethomorph was slightly inferior to metalaxyl and was similar in efficacy to fosetyl-Al at the rates tested.

When applied at the highest rate of 1.2 mg a.i./mL dimethomorph was less effective in controlling *P. cinnamomi* stem infections than at the lower rate of 0.6 mg a.i./mL. The higher concentration was also mildly phytotoxic. This unusual result may be due to fungicide uptake being impaired by the phytotoxic effects, resulting in much lower effective fungicide concentrations within the stem tissues.

All 4 fungicides tested did not eliminate *P. cinnamomi* from deep-seated stem infection in these woody test plants. This result does not mean that under field conditions the same situation would occur. In the field, as the defensive mechanisms of the host are mobilised (Guest 1982) and fungal activity is restricted by unfavourable environment, these fungicides may be able to eliminate *P. cinnamomi* from an established infection or their applications may be timed to prevent infection establishing itself in woody tissues of the root or stem.

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References

British Crop Protection Council (1983). 'Pesticide Manual. A World Compendium.' 7th Edn. (Eds C. R. Worthing and S. B. Wallar.) (British Crop Protection Council, Lavenham Press: Suffolk, G.B.)

Eckert, J. W., and Tsao, P. H. (1962). A selective medium for isolation of *Phytophthora* and *Pythium* from plant roots. *Phytopathology* **52**, 771–7.

Guest, D. I. (1982). Chemically induced host resistance—mode of action of some systemic anti-Oomycete disease controls. Ph.D. Thesis, University of Sydney.

Marks, G. C., Smith, I. W., and Kassaby, F. Y. (1981). Trunk infection of *Eucalyptus* species by *Phytophthora cinnamomi* Rands: a preliminary report. *Australian Forest Research* **11**, 257–67.

Schwinn, F. J. (1983). New developments in chemical control of *Phytophthora*. *In* 'Phtophthora, its Biology, Taxonomy, Ecology and Pathology'. (Eds Erwin, Bartnicki-Garcia and Tsao.) pp. 327–34. (American Phytopathological Society: St Paul Minnesota, USA.)

Shephard, M. C. (1987). Screening fungicides. *Annual Review of Phytopathology* **25**, 189–206.

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Fungi occurring on Proteaceae. I.

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The present study has led to the description of several new fungi occurring on leaves of *Protea* L., *Leucospermum* R.Br., *Telopea* R.Br. and *Brabejum* L. collected from South Africa, Australia or New Zealand. *Cladophialophora proteae* L. Viljoen & Crous, *Coniothyrium nitidae* Crous & S. Denman, *Coniothyrium proteae* Crous & S. Denman, *Coniothyrium leucospermi* Crous & S. Denman, *Harknessia leucospermi* Crous & L. Vilioen, and *Septoria protearum* L. Viljoen & Crous spp. nov. are described from *Protea* and *Leucospermum* in South Africa, while *Phyllosticta owaniana* G. Winter is redescribed from leaves of *Brabejum stellatifolium* L. Furthermore, *Mycosphaerella telopeae* M. Palm & Crous sp. nov. is described from leaves of *Telopea* collected in New Zealand, while *Phyllosticta telopeae* H.Y. Yip, which also occurs on this host, is described in culture from Australian material.

Keywords: *Brabejum, Leucospermum, Protea, Telopea*, fungal systematics.

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Introduction

The Proteaceae, which is the most unique family of the Cape Floral Kingdom, comprises approximately 8600 species, the majority of which are found in the southern hemisphere. About 360 proteaceous species occur in South Africa, of which 330 species in 14 genera are confined to the South-Western Cape Province (fynbos biome) (Rebelo 1995).

Proteaceae, which are grown for cut-flowers in South Africa, form part of a large, economically viable, and expanding industry. Based on a survey conducted during the 1993/1994 season, approximately 2507 ha of veld are cultivated, while an additional 600626 ha are natural fynbos vegetation. Export of fresh fynbos from the Western and Eastern Cape for the 1993/1994 season amounted to nearly R9 million (Malan 1995).

The proteaceous cut-flower industry has considerable potential, but diseases which blemish foliage and blooms are one of the problems which make this a high risk factor crop (Greenhalgh 1981). Since South Africa is the centre of origin for the majority of proteaceous plants, pests and diseases that have evolved with these hosts have become a serious problem not only in South Africa, but to the industry internationally (Knox-Davies 1981). Prior to 1970, the foliicolous fungi occurring on proteas in South Africa were poorly studied (Van Wyk 1973). The description of *Cercostigmina protearum* (Cooke) U. Braun & Crous (= *Cercospora protearum* Cooke) by Cooke (1883), represents the first reference of a leaf pathogen occurring on the genera *Protea, Leucadendron* and *Leucospermum*. However, since the 1970's, numerous diseases of the Proteaceae have been recorded and the causal fungi described (Van Wyk 1973; Van Wyk *et al.* 1975; Benic & Knox-Davies 1983; Van Wyk *et al.* 1985; Knox-Davies *et al.* 1987; Orffer & Knox-Davies 1989; Serfontein & Knox-Davies 1990). Since the Proteaceae are amongst the most endangered species of the Southern African flora, it is in the interest of the conservationist and the Protea industry to record all new diseases and potentially important pathogens. In the present study, nine new fungi, most being associated with leaf spots, are described from leaves of Proteaceae collected in South Africa, New Zealand and Australia.

Materials and methods

Symptomatic leaves and leaf litter samples were incubated in Petri dish moist chambers at 25°C on the laboratory bench to induce sporulation. Single conidium colonies were established on 2% malt extract agar (MEA) (Oxoid), then transferred to plates containing fresh MEA and carnation-leaf agar (CLA) (Fisher *et al.* 1982; Crous *et al.* 1992), and incubated at 25°C under continuous near-ultraviolet light. Linear growth of colonies growing on MEA at 25°C in the dark was measured after 1, 2 or 6 weeks, and colours determined according to the charts of Rayner (1970). Leaf lesions with which species of *Mycosphaerella* were associated were excised and single ascospore cultures established on MEA using the technique described by Crous *et al.* (1991). For microscopic examination the fungi were mounted in lactophenol and measurements made at 1000× magnification. Averages were derived from at least 30 observations, and the ranges are given in parentheses. Reference cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U).

Taxonomy

Cladophialophora proteae *L. Viljoen & Crous* sp. nov., Figures 1, 13, 14.

Conidiogenae cellulae integratae, protuberationes breves truncatas formantes, 2–3 × 1.5–2 µm, mycelio concoloratae, subcylindracea. Conidia in catenis longis acropetalis (ad 20), simplicia vel ramosa, subcylindracea ad oblongo-doliiformia, (9–)13–17(–22) × 2.5–3(–4) µm *in vitro*, 0–1(–2)-septata, pallide brunnea ad pallide olivacea, laevia, hilis subtruncatis ad truncatis, non crassis sed parum refractivis.

Sterile hyphae branched, septate, often forming strands, anastomosing, smooth to finely verruculose, frequently constricted at septa, olivaceous, 3–4 µm wide; hyphal cells in older cultures becoming swollen, up to 6 µm wide. Conidiophores reduced to conidiogenous cells. Conidiogenous cells integrated, forming short, truncate protuberances, 2–3 × 1.5–2 µm, concolorous with mycelium, subcylindrical. Conidia in long acropetal chains (up to 20), simple or branched, subcylindrical to oblong-doliiform, (9–)13–17(–22) × 2.5–3(–4) µm *in vitro*, 0–1(–2)-septate, light brown to pale olivaceous, smooth, hila subtruncate to truncate, not thickened, but somewhat refractive.

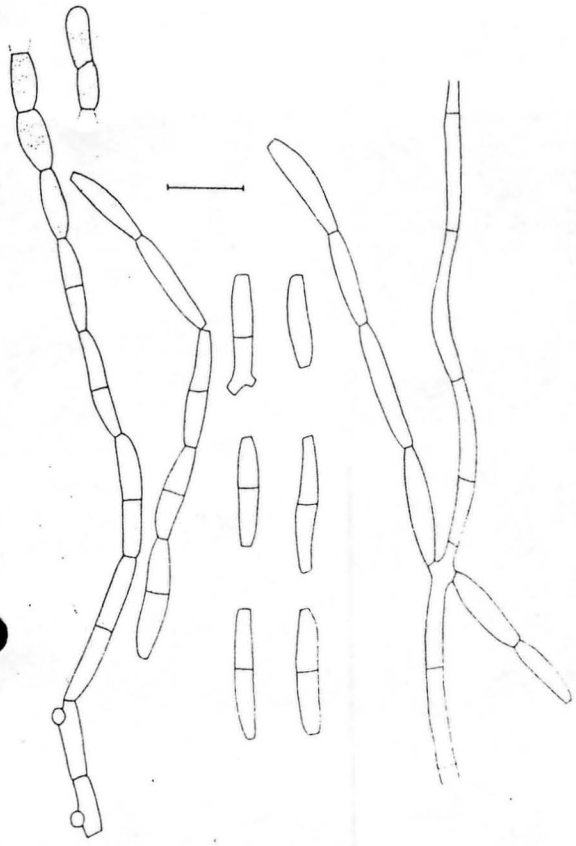


Figure 1 *Cladophialophora proteae*. Chains of 0–1-septate conidia formed on malt extract agar (bar = 10 µm).

Cultural characteristics: Colonies erumpent, segmented, with smooth, sinuate margins; fuscous black 7""k (surface and bottom); aerial mycelium absent. Colonies reaching 5 mm diam. on MEA after 6 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Stellenbósch, J.S. Marias Park isolated as endophyte from leaves of *Protea cynaroides* (L.) L. with *Batcheloromyces* lesions, L. Viljoen, Dec. 1996, PREM 55345 (holotype), cultures ex-type STE-U 1514–1516.

The genus *Cladophialophora* Borelli, which includes species associated with human disorders and others isolated as saprophytes or endophytes from plants, has teleomorphs placed in *Capronia* Sacc. in the Herpotrichiellaceae (Braun & Feiler 1995; De Hoog *et al.* 1995) and *Venturia* Sacc. in the Venturiaceae (Untereiner 1997). Strains isolated as endophytes from leaves of *Protea cynaroides* fit this generic complex. It appears that *Cladophialophora* is heterogenous and it is possible that the saprophytic species will be placed in their own form genus, separate from the human pathogens. The present species is considered congeneric with *Cladophialophora* because of the flat, unthickened but somewhat refractive conidial scars, long conidial chains, and by being saprophytic. Morphologically *C. proteae* is most similar to *C. hachijoensis* (Matsush.) U. Braun & U. Feiler [conidia 1–3-septate, (4.5–)8–25(–35) × (1.5–)2–4(–5) µm)], but *C. proteae* has slightly smaller conidia. Braun and Feiler (1995) depicted a lot of variation between strains presently treated as *C. hachijoensis,* and it is possible that there are even more distinct species within this complex.

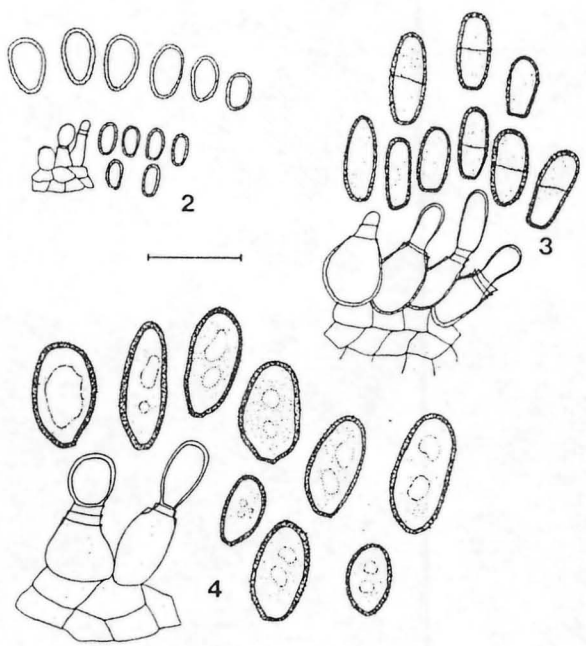
Coniothyrium nitidae *Crous & S. Denman* sp nov., Figures 3, 15.

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, ad 200 µm diam., pariete in ex 3–4 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretae, laeves hyalinae ad pallide olivaceae, doliiformes ad ampulliformes, 1–4 plo enteroblastice et percurrenter proliferantes, 5–8 × 5–10 µm. Conidia pallide ad medio brunnea, parietibus verruculosis, 0–1-septata ellipsoidea ad subcylindracea, apice obtuso, base obtusirotundata ad truncata, (6.5–)8–9(–11) × 3–4(–4.5) µm *in vivo*, (5.5–)6–8(–9) × 3–4 µm *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, medium brown, finely verruculose, 3–4 µm diam. *in vivo*, 2–5 µm *in vitro*, finely verruculose, light to medium brown, forming intercalary and terminal chains of globose chlamydospores. Conidiomata pycnidial, subepidermal, globose, separate, brown, up to 200 µm diam., wall consisting of 3–4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline to pale olivaceous, doliiform to ampulliform, proliferating 1–4 times enteroblastically and percurrently, 5–8 × 5–10 µm. Conidia medium brown, thick-walled, verruculose, 0–1-septate, ellipsoidal to subcylindrical, apex obtuse, base bluntly rounded to truncate, (6.5–)8–9(–11) × 3–4(–4.5) µm *in vivo*, (5.5–)6–8(–9) × 3–4 µm *in vitro*.

Cultural characteristics: Colonies with irregular margins; grey olivaceous 21""b to cinnamon 13"b (bottom); aerial mycelium moderate, dirty pink to white. Colonies reaching 32 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Hermanus, leaves of *Protea nitida* Mill., S. Denman, 29 Aug. 1996, PREM 55346 (holotype), cultures ex-type STE-U 1476–1478, 1531–1533.



Figures 2–4 Conidia and conidiogenous cells of *Coniothyrium* spp. *in vivo*. 2. *C. proteae*. 3. *C. nitidae*. 4. *C. leucospermi*. (bar = 10 µm).

Coniothyrium proteae *Crous & S. Denman* sp. nov., Figures 2, 16.

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, 60–120 µm in diam., pariete ex 2–3 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretae, laeves hyalinae, doliiformes ad ampulliformes 1–2 plo enteroblastice et percurrenter proliferantes, 3–8 × 3–4 µm. Conidia pallide ad medio brunnea, parietibus tenuibus, laevia ad subtiliter verruculosa, aseptata, ellipsoidea ad globosa, rare pyriformia, apice obtuso, base obtusirotundata ad truncate, (5–)5.5–7(–8) × 3.5–4 µm *in vivo*, (3–)3.5–4 × 2–2.5 µm *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, branched, pale to medium brown, smooth to finely verruculose, 3–4 µm diam. Conidiomata pycnidial, subepidermal, globose, separate, brown, 60–120 µm diam., wall consisting of 2–3 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline, doliiform to ampulliform, proliferating 1–2 times enteroblastically and percurrently, 3–8 × 3–4 µm. Conidia light to medium brown, thin-walled, smooth to finely verruculose, aseptate, ellipsoidal to globose, rarely pyriform, apex obtuse, base bluntly rounded to truncate, (5–)5.5–7(–8) × 3.5–4 µm *in vivo*, (3–)3.5–4 × 2–2.5 µm *in vitro*.

Cultural characteristics: Colonies with smooth, sinuate margins, olivaceous grey 23""i (bottom), smoke grey 21""f (surface); aerial mycelium sparse to moderate. Colonies reaching 4 mm diam. on MEA after 2 weeks in the dark at 25°C.

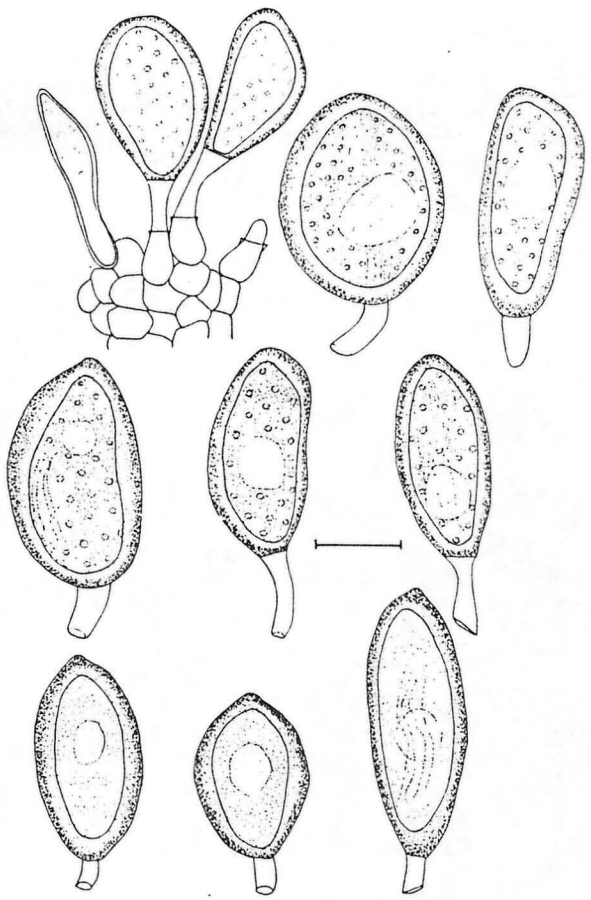


Figure 5 *Harknessia leucospermi*. Conidia and conidiogenous cells *in vivo* (bar = 10 µm).

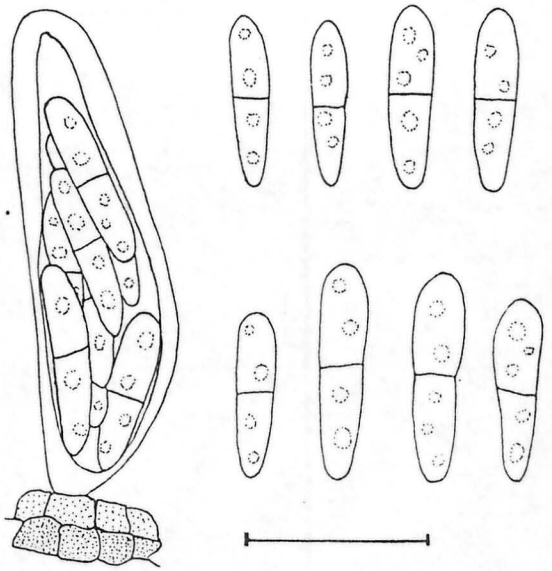


Figure 6 *Mycosphaerella telopeae*. Ascospores and ascus *in vivo* (bar = 10 µm).

Specimens examined: South Africa, Western Cape Province, Hermanus, leaves of *Protea nitida*, S. Denman, 29 Aug. 1996, PREM 55347 (holotype), cultures ex-type STE-U 14231425; Western Cape Province, Stellenbosch, leaves of *Protea mellifera*, L. Verwoerd, Apr. 1923, BPI 639096.

Coniothyrium leucospermi *Crous & S. Denman* sp. nov., Figures 4, 17.

Conidiomata pycnidialia, subepidermalia, amphigena, discreta, globosa atrobrunnea ad 200 µm in diam., pariete ex 3–4 stratis cellularum brunnearum texturae angularis. Conidogenae cellulae discretae, laeves, pallide brunneae, doliiformes ad ampulliformes, 1–3 plo enteroblastice et percurrenter proliferantes, 9–11 × 5–7 µm. Conidia mediobrunnea, parietibus crassis, verruculosa, aseptata, ellipsoidea ad globosa, apice obtuso, base obtusirotundata ad truncate, 11–13 × 5–6 µm *in vivo*, (9–)10–13(–15) × 6–7 µm *in vitro*.

Leaf spots amphigenous, irregular, grey to light brown with a raised, dark brown border, frequently associated with tip blight of leaf margins. Conidiomata pycnidial, subepidermal, amphigenous, separate, globose, dark brown, up to 200 µm diam., wall consisting of 3–4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, light brown, doliiform to ampulliform, proliferating 1–3 times enteroblastically and percurrently, 9–11 × 5–7 µm. Conidia medium brown, thick-walled, verruculose, aseptate, ellipsoidal to globose, apex obtuse, base bluntly rounded to truncate, 11–13 × 5–6 µm *in vivo*, (9–)10–13(–15) × 6–7 µm *in vitro*.

Cultural characteristics: Colonies with smooth, regular margins, fucous black 7""k (surface), olivaceous black 27""m (bottom); aerial mycelium sparse. Colonies reaching 12–16 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined: South Africa, Western Cape Province, Piketberg, leaves of *Leucospermum conocarpodendron* (L.) H. Buek., S. Denman, 29 Aug. 1996, PREM 55348 (holotype), cultures ex-type STE-U 1426–1428. Dominican Republic, leaves of *Leucospermum* sp., L. Schroeder, 7 Jul. 1986, BPI 1107823.

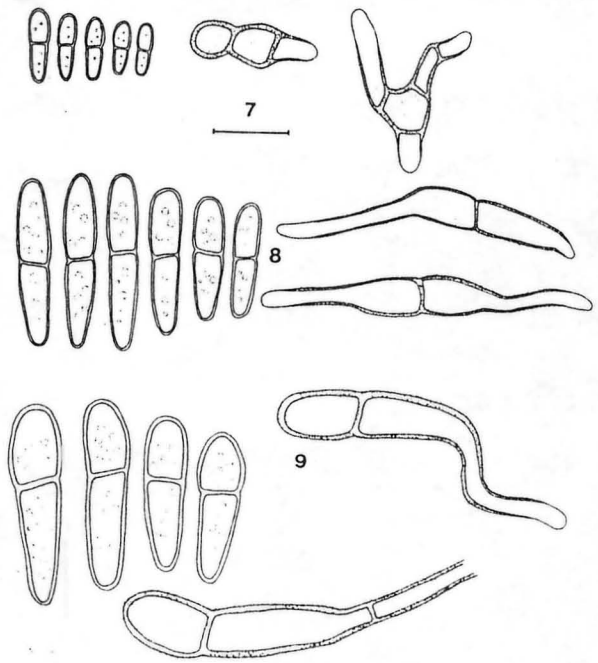


Figure 7–9 Ascospores and germinating ascospores of *Mycosphaerella* spp. on malt extract agar after 24 h. 7. *M. belulus*. 8. *M. jonkershoekensis*. 9. *M. protea*. (bar = 10 µm).

Van Wyk (1973) listed several specimens from *Protea* and *Leucadendron* (PREM 44798, 44853, 44854) which he considered to represent a new species of *Coniothyrium*. An examination of these specimens found conidiomata to be present on PREM 44854 and 44798. Conidia were aseptate, finely verruculose, and resembled *C. protea* in shape, but were much larger [(7–)8–9(–10) × 4–5(–6) in PREM 44854; (5–)7–8(–9) × (2.5–)4–5(–6) in PREM 44798]. Although it appears that these collections represent yet another distinct species, further collections and cultures are required to suitably characterise this pathogen.

As far as we could establish, only one other species of *Coniothyrium*, namely *C. proteae-abyssinicae* Bacc. has been described from these hosts. The latter species has conidia that differ in size (14.4 × 3.2 µm; Baccarini 1917) to those of the species described in the present study. It is important to note, however, that major differences occurred in conidial shape and dimension in some species when cultured on agar, and these discrepancies will have to be carefully considered when comparing new species and isolates in the future.

Harknessia leucospermi *Crous & L. Viljoen* sp. nov., Figures 5, 18.

Conidiomata discreta, immersa, globosa ad subglobosa, uniloculata, subepidermalia, ad 350 µm diam. Conidiogenae cellulae subcylindraceae ad lageniformes, hyalinae, laeves, 8–20 × 2.5–5 µm. Conidia holoblastica, late ventricosa, guttula media, granularia, laevia, irregulariter striata, apice obtusa ad obtusirotundato, base truncata (23–)25–28(–32) × (13–)15– 17(–18) µm, appendicula basali hyalina non ramosa 4–8(–14) × 2–2.5(–3) µm.

Conidiomata separate, immersed, globose to subglobose, unilocular, subepidermal, up to 350 µm diam, ostiole with light brown furfuraceous margin; basal and lateral walls 5–7 cells thick composed of *textura angularis*, brown, becoming hyaline towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells subcylindrical to lageniform, hyaline, smooth, 8–20 × 2.5–5 µm. Conidia holoblastic, broadly ventricose with a central guttule, granular, smooth, irregularly striate, apex obtuse to bluntly apiculate, base truncate (23–)25–28(–32) × (13–)15–17(–18) µm, with a hyaline, unbranched basal appendage 4–8(–14) × 2–2.5(–3) µm *in vivo*; conidia broadly ellipsoid, apiculate, (23–)25–27(–30) × (12–)13–15 µm, basal appendage 3–10 × 2–2.5 µm *in vitro*.

Colony characteristics: Colonies with moderate, pale yellow aerial mycelium, luteus 21b (bottom); margins smooth to irregular. Colonies reaching 56 mm diam. on MEA after 1 week in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Kirstenbosch, leaf litter of a *Leucospermum* sp., P.W. Crous, 20 May 1996, PREM 55349 (holotype), cultures ex-type STE-U 1372-1374, IMI 375227, ATCC 201156, CBS 778.97.

Harknessia leucospermi is morphologically similar to *H. eucalypti* Cooke apud Cooke and Harkn., which has broadly ventricose, apiculate conidia (19–)2–25(–28) × (11–)13–15 µm with restricted striations and basal appendages (6–)8–13 × 2–4 µm, and *H. eucalyptorum* Crous *et al.*, which has broadly ventricose, astriate conidia with blunt apices, (16–)20–25(–29) × (9–)10–14(–16) µm *in vivo*, (14.5 -)17–22(–24) × (10.5–)11–13(–14) µm *in vitro*, basal appendages 3–16 µm (Crous *et al.* 1993).

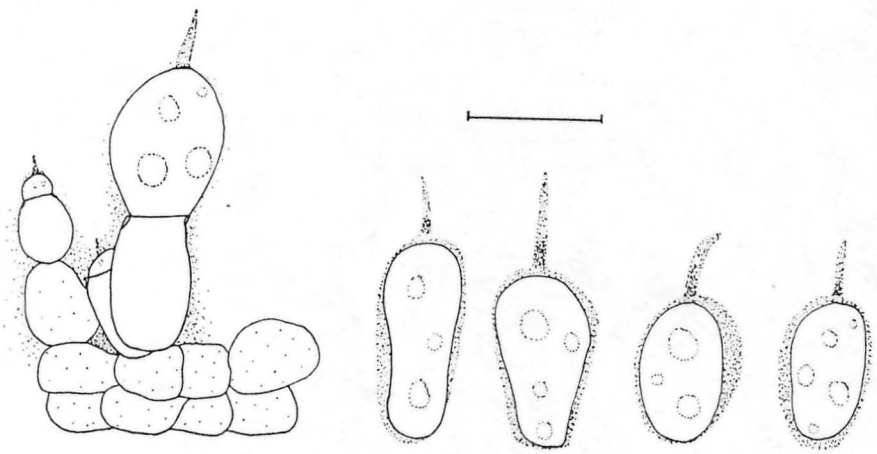


Figure 10 *Phyllosticta owaniana* sporulating on malt extract agar (bar = 10 µm).

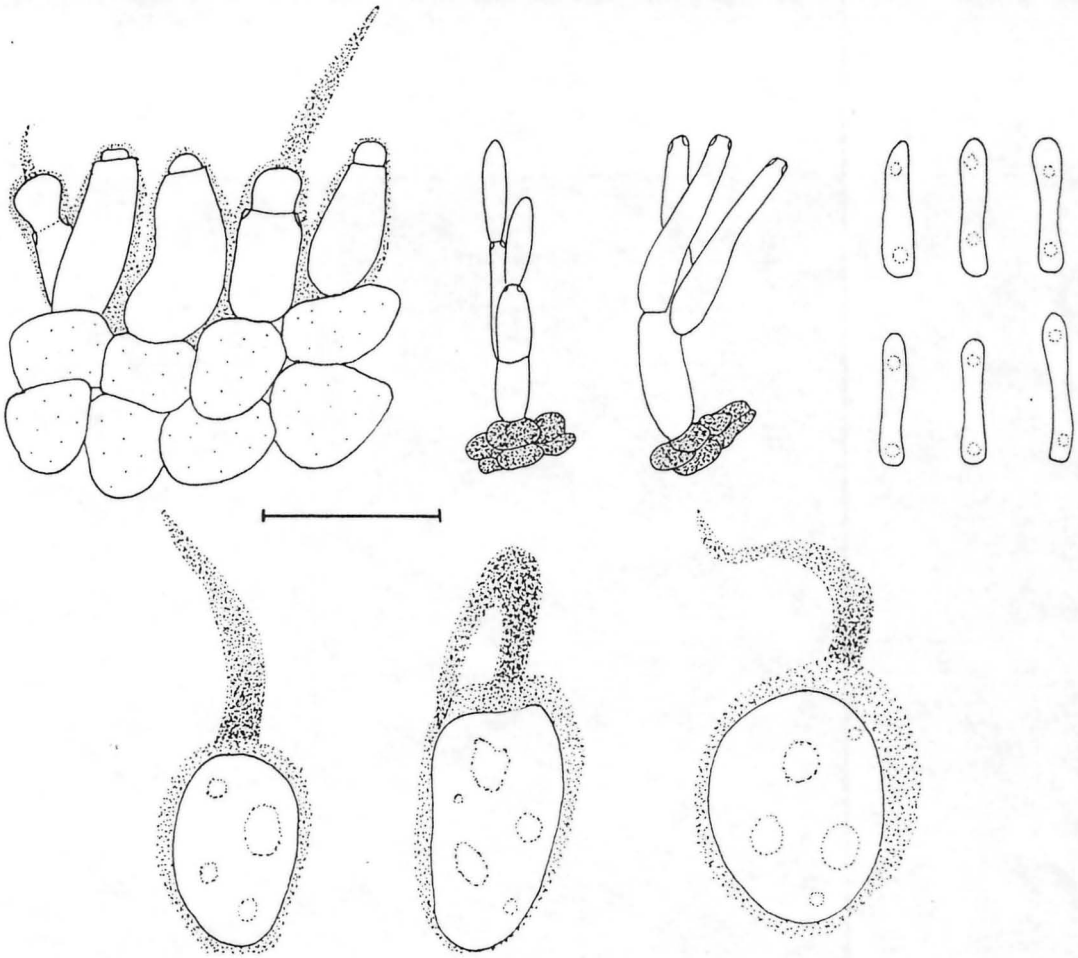


Figure 11 *Phyllosticta telopeae* sporulating on malt extract agar (bar = 10 µm).

Sutton and Pascoe (1989) reported *H. eucalypti* occurring on *Banksia marginata* Cav. and *Lambertia formosa* Sm., both hosts in the Proteaceae. Conidia of *H. leucospermi* are larger than those of *H. eucalypti* and *H. eucalyptorum*. On leaf tissue the conidia resemble those of *H. eucalyptorum* in being more bluntly apiculate. In culture, however, conidia become more broadly ellipsoidal and sharply apiculate, distinct from conidia of *H. eucalyptorum* formed in culture (Crous *et al.* 1993). Although Nag Raj (1993) illustrated conidia of *H. eucalypti* to be striate, striations were only observed in restricted areas on some conidia, and were not as prominent as in *H. leucospermi*. The latter feature was also found to be constant in culture for *H. leucospermi*.

Mycosphaerella telopeae *M. Palm & Crous* sp. nov., Figure 6.

Pseudothecia amphigena, sparse distributa, unica, nigra, erumpentia globosa ad 120 µm diam. Asci aparaphysati fasciculati bitunicati subsessiles obovoidei ad late ellipsoidei vel cylindracei, recti vel parum curvati, 8 sporis, 20–28 × 8–10 µm. Ascosporae multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, fusoideo-ellipsoideae apicibus obtusis, latissimae in medio cellulae apicalis, mediano 1-septatae, magis prominanter ad basim contractae (9–)10–11(–12) × (2–)2.5(–3) µm.

Leaf spots circular, amphigenous, 1–4 mm diam., grey in centre, surrounded by a raised, dark brown border and a narrow chlorotic margin. Pseudothecia amphigenous, sparsely distributed, single, black, erumpent, globose, up to 120 µm diam.; apical papillate ostiole 5–10 µm in diam.; wall consisting of 3–4 layers of medium brown *textura angularis*. Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid or cylindrical, straight or slightly curved, 8-spored, 20–28 × 8–10 µm. Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, medianly 1-septate, generally not constricted at septum, with some ascospores on the leaf surface appearing slightly constricted; ascospores tapering more prominently towards the lower end (9–)10–11(–12) × (2–)2.5(–3) µm.

Specimens examined: New Zealand, leaf of *Telopea* sp., M. Abdelshife, 11 Sept. 1996, PREM 55350 (holotype); New Zealand, leaf of *Telopea* sp., M. Abdelshife, 5 Aug. 1996, BPI 806263; New Zealand, leaf of *Telopea* sp., M. Abdelshife, 6 Aug. 1996, BPI 806264.

Although no species of *Mycosphaerella* has been described from *Telopea* (Corlett 1991, 1995), three species are presently known from leaves of *Protea* (Proteaceae), namely *M. proteae* (Syd.) Arx, *M. jonkershoekensis* Van Wyk *et al.* and *M. bellulus* Crous and M.J Wingf. (Figures 7–9) (Crous & Wingfield 1993). Ascospores of *M. telopeae* are much smaller than those of the large-spored *M. proteae*, which measure 20–33 × 6–8 ($\bar{x}$ = 26 × 7) µm. Morphologically ascospores of *M. telopeae* resemble those of *M. jonkershoekensis* which measure 11–23 × 4–6 ($\bar{x}$ = 18 × 4.5) µm, but ascospores of the former differ in being much smaller and less prominently constricted at the septum. Ascospores of *M. telopea* are slightly larger than those of *M. bellulus*, 7–11 × 2–3 ($\bar{x}$ = 9 × 2.5) µm, and are not as

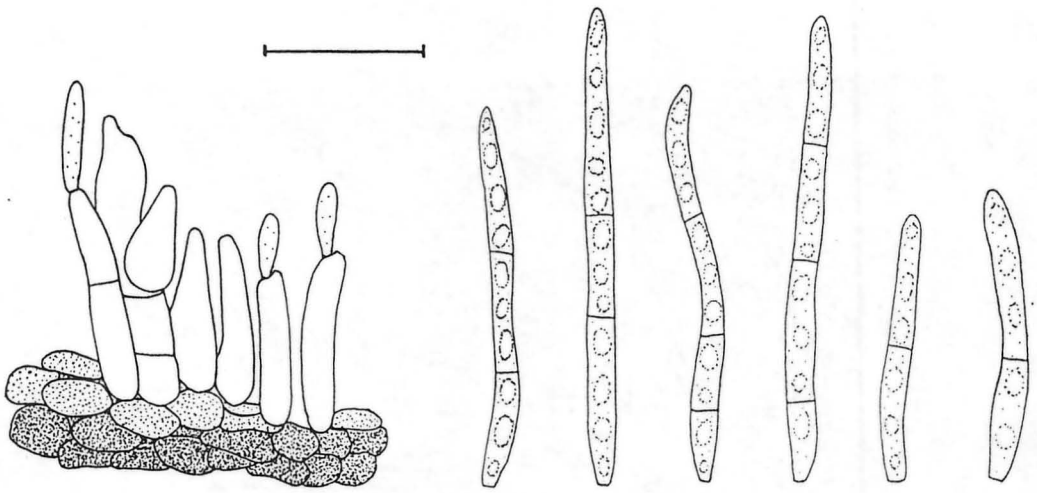


Figure 12 *Septoria protearum*. Conidia and conidiogenous cells formed on carnation leaf agar (bar = 10 µm).

prominently constricted as in the latter species. The erumpent black pseudothecia of *M. telopea* are also distinct from those of *M. jonkershoekensis* and *M. bellulus*, which are subepidermal and generally not visible to the naked eye.

When Crous and Wingfield (1993) treated the species of *Mycosphaerella* occurring on *Protea*, little was known about their behaviour in culture. Subsequent to that study, fresh collections were obtained of all three of those species. As observed in the type collection of *M. jonkershoekensis* (PREM 44830), ascospores from fresh collections were frequently slightly olivaceous in their asci. When ascospores are shot out for germination on MEA (Crous *et al.* 1991), ascospores become verruculose, brown, and constricted at the septum. Ascospores germinate initially with germ tubes growing parallel to the long axis of the spore (Figure 8). After 48 h, however, ascospores have usually formed several germ tubes, and the germination is irregular. A peculiarity about *M. jonkershoekensis* is that ascospores germinate at 25°C, but die soon after germination if the plates are not incubated at 15°C for one to two weeks. After this initial phase the fungus will grow at most temperatures, and it is hypothesised that this low temperature requirement is a prerequisite for successful germination and infection of leaf tissue. The same phenomenon has also recently been reported for *M. juvenis* Crous and M.J. Wingf. on *Eucalyptus* (Crous & Wingfield 1996).

Ascospores of *M. bellulus* germinate with one to several germ tubes which grow irregularly to the long axis of the spore. As with *M. jonkershoekensis*, spores darken and become verruculose at germination (Figure 7). In the present study, *M. bellulus* was also isolated from leaf lesions of *Leucospermum* spp. (STE-U 1321–1323), and it appears to be a very common species of *Mycosphaerella* on *Protea* spp., frequently also occurring in association with *Leptosphaeria protearum* Syd.

After several unsuccessful attempts, ascospores of *M. proteae* were finally induced to germinate in culture. Unlike *M. jonkershoekensis* and *M. proteae*, ascospores could never be induced to shoot out onto the agar surface, and the epidermis had to be cut open to expose the pseudothecia. This difference, as well as the distinct lesions and red-purple discolouration of the leaf tissue suggest that *M. proteae* is a fungus quite unrelated to the other species dealt with above. In culture, germinating ascospores become constricted at their septum, brown in colour, and germinate with one germ tube generally parallel to the long axis of the spore (Figure 9). Ascospores did not become as verruculose as those of *M. bellulus* and *M. jonkershoekensis*. Colonies were extremely slow growing, and after about 6 months at 25°C on MEA had hardly reached 5 mm in diam., suggesting that this fungus is more of an obligate pathogen than the other species of *Mycosphaerella* treated here.

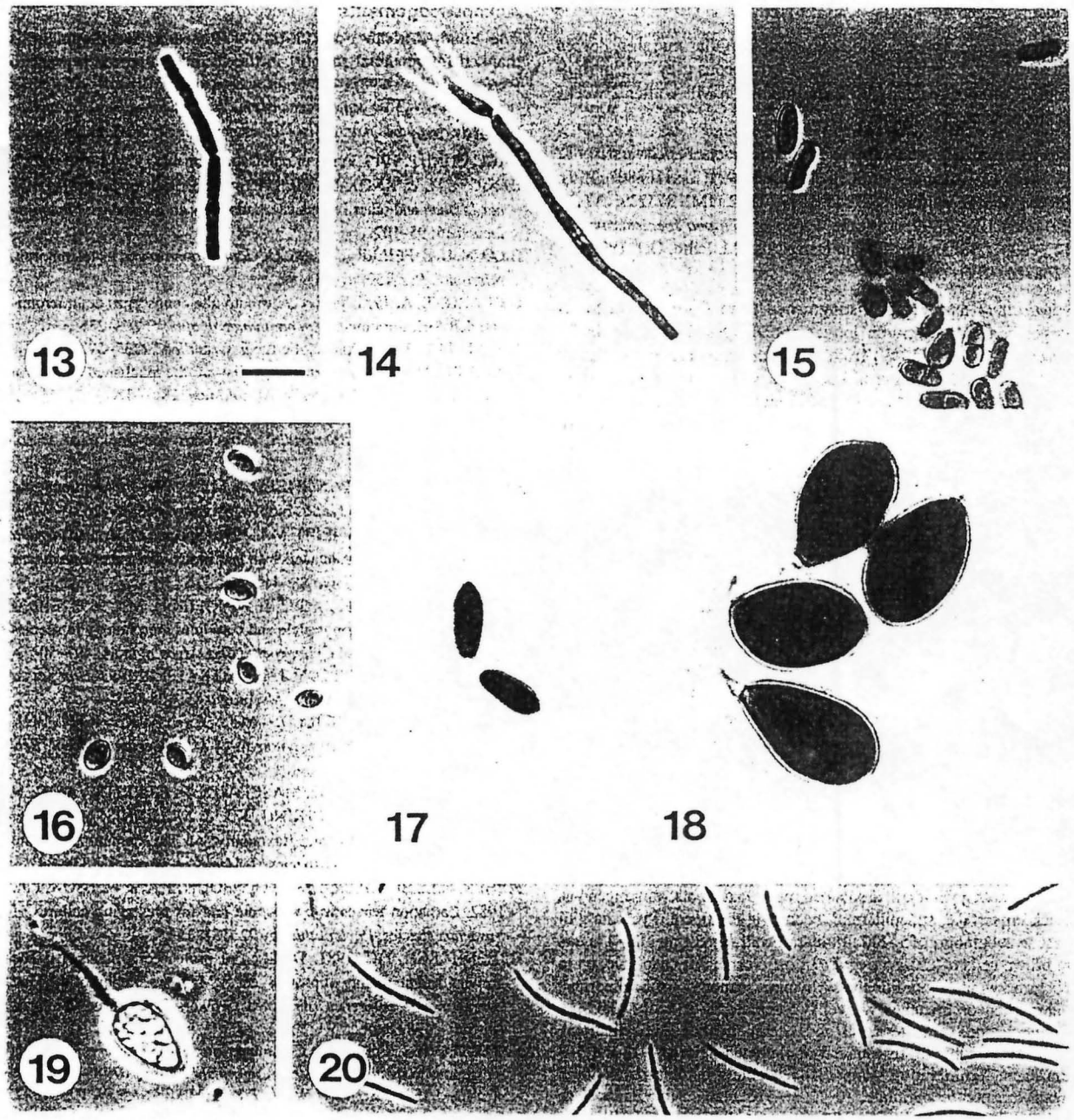
Phyllosticta owaniana G. Winter, Hedwigia 24: 3 1(1885) Figure 10.

Leaf spots amphigenous, circular, 0.5–5 mm diam., light brown, becoming darker brown towards the raised, dark brown border; margins chlorotic when present. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye, scattered, immersed, becoming erumpent, globose to subglobose, up to 120 µm diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 µm thick, of *textura angularis*, with brown cells becoming lighter towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth-walled, subcylindrical to doliiform, 5–8 × 3–6 µm. Conidia obovoid to ovoid *in vivo*, ovoid *in vitro*, apex rounded, base truncate to rounded, hyaline, guttulate, (10–)12–14(–15) × 7–8(–9) µm *in vivo* and *in vitro*, enclosed in a mucous sheath 0.5–3 µm thick, persistent on most conidia, bearing a single, unbranched, slightly tapering apical mucoid appendage 5–8(–14) × 1–1.5 µm.

Cultural characteristics: Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous black 27""m (bottom). Colonies reaching 9.5 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Jonkershoek leaf spots on *Brabejum stellatifolium* L., A. den Breeÿen, Mar. 1995, PREM 55351, cultures STE-U 1009–1010, IMI 375228, ATCC 201157, CBS 776.97.

Phyllosticta owaniana appears to be a well-established pathogen of *Brabejum stellatifolium* and is associated with prominent leaf spots on this plant throughout the Western Cape, where this host is planted as an ornamental. The present collection correlates well with the original description of Winter (1885), who cited conidia as being ovoid to subpyriform, 10–12 × 8 µm.



Figures 13–20 Conidia of microfungi occurring on Proteaceae. **13, 14.** Catenulate conidia of *Cladophialophora proteae*. **15.** *Coniothyrium nitidae*. **16.** *Coniothyrium proteae*. **17.** *Coniothyrium leucospermi*. **18.** *Harknessia leucospermi*. **19.** *Phyllosticta telopeae*. **20.** *Septoria protearum* (bar = 10 µm).

Phyllosticta telopeae H. Y. Yip, Mycol. Res. 93: 494 (1989), Figures 11, 19.

Leaf spots amphigenous, circular to somewhat irregular, often confined by leaf veins, 2–7 mm diam., grey-brown to grey olivaceous with a narrow, dark, slightly raised border on the adaxial surface. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye, scattered, immersed, becoming erumpent, globose to subglobose, up to 150 µm diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 µm thick of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells hyaline, smooth-walled, subcylindrical to lageniform, 7–12 × 3–5 µm, often proliferating once enteroblastically and percurrently. Macroconidia ellipsoidal to obovoid with a rounded apex and a truncate base, rarely with a minute marginal frill, unicellular, hyaline, smooth-walled, guttulate, (12–)13–16(–18) × (7–)8–9 µm *in vitro*, 11.5–15.5 × 7–10 µm *in vivo*, enclosed in a thin mucoid sheath, 0.5–2 µm thick bearing a single, unbranched, attenuated apical mucoid appendage 10–20 × 2–3 µm on MEA (up to 40 µm long when cultured on a medium consisting of 2% malt extract, 2% V8 juice and 4% agar), (6.5–)20–100 × 2–3 µm *in vivo*. Microconidiophores subcylindrical, hyaline, 0–2-septate, branched above, 15–20 × 2–3 µm. Microconidiogenous cells subcylindrical, ampulliform to lageniform with minute periclinal thickening, hyaline, smooth-walled, 5–12 × 2–2.5 µm. Microconidia bacillar with a rounded apex and swollen, truncate base, unicellular, hyaline,

smooth-walled, (6–)8–10(–12) × 1.5 µm.

Colony characteristics: Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous grey 25""m (bottom). Colonies reaching 35.2 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined: Australia, leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi, det. M. Palm, Oct. 1991, US1108807 (BPI), PREM 55352, cultures STE-U 1517, 1522, IMI 375229, ATCC 201158, CBS 777.97; Australia, leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi & J. Van Dersal, det. M. Palm, Oct. 1997, US 1108820 (BPI).

Phyllosticta telopeae is distinguished from *P. owaniana* by its larger conidia and much longer appendages. It was originally described by Yip (1989) from *T. speciosissima* leaves collected in Tasmania. Our material correlates well with the description given by Yip, with the only difference being the length of the mucoid appendages. The fact that appendages were observed to vary in length depending on the medium on which they were cultured, once again stresses the importance of standardising conditions and media when comparing species and descriptions of *Phyllosticta*. As far as we are aware, this is the first description of *P. telopeae* from culture, and the first record of its microconidial state.

Septoria protearum L. Viljoen & Crous sp nov., Figures 12, 20.

Conidiomata pycnidialia, globosa ad subglobosa, 65–200 µm diam. Conidiophorae hyalinae, laeves, subcylindraceae, nonramosae vel superne ramosae, 0–5-septatae, 8–30 × 1.5–3.5 µm. Conidiogenae cellulae terminales et laterales, hyalinae, subcylindraceae, non ramulosae, ad apices rotundatas planes contractae, 4–12 × 1.5–3 µm diam.; sympodialiter proliferantes. Conidia holoblastica, solitaria hyalina laevia guttulata, (0–)1–3(–4)-septata, subcylindracea ad anguste obclavata, apice subobtuso, base angusta, obconico-truncata ad truncata, recta ad curvata (6–)15–22(–30) × 1.5–2 µm *in vitro*.

Conidiomata pycnidial, associated with leaf spots, amphigenous, black on surface, subepidermal, becoming erumpent. Pycnidia globose to subglobose, 65–200 µm diam.; wall consisting of 3–4 layers of brown cells of *textura angularis*; ostioles slightly papillate, up to 60 µm wide. Conidiophores hyaline, smooth, subcylindrical, unbranched or branched above, 0–5-septate, 8–30 × 1.5–3.5 µm. Conidiogenous cells terminal and lateral, hyaline, subcylindrical, unbranched, tapering to rounded or flattened apices, 4–12 × 1.5–3 µm diam., proliferating sympodially. Conidia holoblastic, solitary, hyaline, smooth, guttulate, (0–)1–3(–4)-septate, subcylindrical to narrowly obclavate, apex subobtuse, base narrow obconically truncate to truncate, straight to curved, (6–)15–22(–30) × 1.5–2 µm *in vitro*.

Cultural characteristics: Colonies with smooth margins, iron grey 23""k (bottom), with moderate whitish aerial mycelium. Colonies reaching 10.3 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Gauteng Province, Pretoria, leaves of *Protea cynaroides*, L. Viljoen, Sept. 1996, PREM 55353 (holotype), culture ex-type STE-U 1470, IMI 375230, ATCC 201159, CBS 778.97.

As far as we could establish, only one other species of *Septoria* has thus far been associated with leaf spots on, or described from leaves of *Protea*. *Septoria proteae* Ciccar. was described as having 1–3-septate conidia, 40–50 × 3–4 µm (Ciccarone 1951), thus being much larger than those of *S. protearum*.

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References

BACCARINI, P. 1917. Fungi etiopici. *Ann.Bot.* 14: 117–140.

BENIC, L.M. & KNOX-DAVIES, P.S. 1983. Scab of *Leucospermum cordifolium* and other Proteaceae, caused by an *Elsinoë* sp. *Phytophylactica* 15: 95–107.

BRAUN, U. & FEILER, U. 1995. *Cladophialophora* and its teleomorph. *Microbiol. Res.* 150: 81–91.

CICCARONE, A. 1951. Primo contributo alla conoscenza dei micromiceti dell'Africa orientale. *Mycopath mycol. appl.* 5: 208–235.

COOKE, M.C. 1883. Some exotic fungi. *Grevillea* 12: 37–39.

CORLETT, M. 1991. An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. *Mycol.Mem.* 18: 1–328.

CORLETT, M. 1995. An annotated list of the published names in *Mycosphaerella* and *Sphaerella*: corrections and additions. *Mycotaxon* 53: 37–56.

CROUS, P.W. & WINGFIELD, M.J. 1993. Additions to *Mycosphaerella* in the fynbos biome. *Mycotaxon* 46: 19–26.

CROUS, P.W. & WINGFIELD, M.J. 1996. Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. *Mycologia* 88: 441–458.

CROUS, P.W., PHILLIPS, A.J.L. & WINGFIELD, M.J. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* 84: 497–504.

CROUS, P.W., WINGFIELD, M.J. & NAG RAJ, T.R. 1993. *Harknessia* spp. occurring in South Africa. *Mycologia* 85: 108–118.

CROUS, P.W., WINGFIELD, M.J. & PARK, R.F. 1991. *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. *Mycol. Res.* 95: 628–632.

DE HOOG, G.S., GUÉHO, E., MASCLAUX, F., GERRITS VAN DEN ENDE, A.H.G., KWON-CHUNG, K.J. & MCGINNIS, M.R. 1995. Nutritional physiology and taxonomy of the human-pathogenic *Cladosporium-Xylohypha* species. *J. Med. Vet. Mycol.* 33: 339–347.

FISHER, N.L., BURGESS, L.W., TOUSSOUN, T.A. & NELSON, P.E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

GREENHALGH, F.C. 1981. Diseases of proteaceous plants. In: The growing and marketing of proteas, ed. P. Mathews. Report of the First International Conference of Protea Growers, Melbourne, Victoria, Australia, 4–8 October.

KNOX-DAVIES, P.S. 1981. Comments on fungus diseases of plants indigenous to the South Western Cape. *Veld & Flora* 67: 88–91.

KNOX-DAVIES, P.S., VAN WYK, P.S. & MARASAS, W.F.O. 1987. Diseases of *Protea*, *Leucospermum* and *Leucadendron* recorded in South Africa. *Phytophylactica* 19: 327–337.

MALAN, G. 1995. The fynbos ornamental industry: 1993–1995. Unpublished report of the Fynbos Unit, ARC, Elsenburg, Western Cape, South Africa.

NAG RAJ, T.R. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Waterloo, Ontario, Canada.

ORFFER, S. & KNOX-DAVIES, P.S. 1989. A canker and die-back disease of *Protea repens*. *Phytophylactica* 21: 189–194.

RAYNER, R.W. 1970. A mycological colour chart. CMI and British Mycological Society. Kew, Surrey, England.

REBELO, T. 1995. Proteas: a field guide to the proteas of southern Africa. Fernwood Press, Vlaeberg, South Africa.

SERFONTEIN, S. & KNOX-DAVIES, P.S. 1990. Leaf spot of *Protea magnifica* and copper leaf of *Leucospermum cordifolium* associated with *Coleroa senniana*. Phytophylactica 22: 103–107.

SUTTON, B.C. & PASCOE, I.G. 1989. Addenda to *Harknessia* (Coelomycetes) *Mycol. Res.* 92: 431–439.

UNTEREINER, W.A. 1997. Taxonomy of selected members of the ascomycete genus *Capronia* with notes on anamorph-teleomorph connections. *Mycologia* 89: 120–131.

VAN WYK, P.S. 1973. Funguspatogene van die genera *Protea, Leucadendron* en *Leucospermum* met spesiale verwysing na *Phytophthora cinnamomi*. Ph.D. thesis, University of Stellenbosch, Stellenbosch, South Africa.

VAN WYK, P.S., MARASAS, W.F.O. & KNOX-DAVES, P.S. 1975. Ascomycetous leaf pathogens of *Protea*, *Leucadendron* and *Leucospermum* in South Africa. Phytophylactica 7: 91–94.

VAN WYK, P.S., MARASAS, W.F.O. & KNOX-DAVIES, P.S. 1985. *Batcheloromyces leucadendri* sp. nov. on *Leucadendron* spp. in South Africa. *S. Afr. J. Bot.* 51: 344–346.

WINTER, G. 1885. Exotische Pilze II. *Hedwigia* 24: 21–35.

YIP, H.Y. 1989. Five new species of *Phyllosticta* on Australian native plants. *Mycol. Res.* 93: 489–496.

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Yield and seasonal growth flushing of *Protea* 'Pink Ice' and *Leucadendron* 'Silvan Red' in South Australia

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Summary. Data on yield and growth flushing of 2 protea varieties were collected at commercial sites in South Australia over 3 years. Mean (± s.e.) yield of *Protea* 'Pink Ice' in terms of marketable stems averaged 63 (± 7) stems/plant on a highly fertile soil to 39 (± 1) stems/plant on an infertile site. On the same sites, *Leucadendron* 'Silvan Red' yielded means (± s.e.) of 314 (± 13) and 219 (± 5) marketable stems/plant. Data are presented in marketing classes based on stem length. Monthly increases in stem length and diameter were used to determine seasonal growth flushing patterns in the 2 cultivars. Pink Ice commenced annual growth in August–September, reached peak growth rate in October and fell to low levels in December. Silvan Red commenced growth between October and November, reached peak growth rate in December and continued stem elongation until March when all growth ceased until the following year.

Introduction

The Australian *Protea* Industry has developed in size between 1980 and 1994 to a planted area of about 1200 ha (Yencken 1994). This industry is based on many species and cultivars of South African and Australian origin grown on a wide range of soil types. Yield performance of *Protea* species and cultivars is largely anecdotal. Without yield guidelines, growers have difficulty in assessing the performance of their plantings, determining maintenance fertiliser management programs and evaluating the economics of substituting varieties as the market changes.

When this research commenced, *Protea* 'Pink Ice' and *Leucadendron* 'Silvan Red' were the 2 most widely planted *Protea* cultivars in Australia. *Protea* Pink Ice is a hybrid of *P. neriifolia* and *P. compacta* which was released to the industry in 1981, and has been particularly successful due to its vigorous habit and tolerance to disease. *Leucadendron* 'Silvan Red' (*L. laureolum* x *L. salignum*) has been a widely grown, high yielding variety in South Australian conditions although its popularity has been recently superseded by a similar cultivar, *Leucadendron* 'Safari Sunset'. Strong hybrid vigor is expressed as a rule with interspecific crosses in *Proteas* (Brits 1992), with common yield increases of 100–150%.

There are few studies which report yield data of cultivated proteas. Fuss and Sedgley (1991) assessed production from seedling *Banksia* plants at 6 commercial sites and found significant between-plant variation in yield between plants within sites and between sites. In *P. neriifolia* 'Salmon Pink' (Dupee and Goodwin 1990), significant differences in flower number and flowering date were observed at 4 commercial sites, however, total yield on a per plant basis was not reported.

Growth flushing has been studied in *Protea* species principally in relation to time of flowering and to understand site-dependant seasonal growth patterns. In South Africa, *P. neriifolia* 'Kouga' cultivated in plantations showed peak vegetative growth in August and September (early spring) with a secondary growth flush in January–February (Heinsohn and Pammenter 1988). Flowering occurred from January to July with peak yields in April and May. *Protea neriifolia* Salmon Pink showed a minor growth flush in October in New South Wales (Dupee and Goodwin 1992) with the main vegetative peak in January–February. In the same study, seedlings of King protea, *P. cynaroides*, showed 2 growth flushes each year (October and March), although the amount of vegetative growth differed markedly over the 2 years of the study.

Leaf analysis surveys of *Protea* species conducted throughout Australia (Barth 1994) demonstrated that the variability between individuals within most species grown in commercial plantations (of seedling origin) made interpretation of leaf nutrient values difficult. Distinct seasonal changes in leaf analysis values also emerged in this study which highlighted the importance of coordinating the growth status of plants with leaf sampling times.

It was the aim of this study to establish baseline data on yields achieved under commercial conditions for

2 important *Protea* cultivars grown in South Australia. Data were collected at 3 sites representing variations in soil type and nutrient composition. Yield was assessed in terms of commercial quality flower stems (graded by stem lengths reflecting marketing practices), unmarketable stems, damaged material and prunings. Stem growth was monitored monthly to define growth flushing patterns which can be used to determine optimal periods for fertiliser application and leaf nutrient monitoring. This information was used to assist in the interpretation of seasonal trends in leaf analysis values obtained as part of a nutrient management research program (Maier *et al.* 1995). Values obtained from clonal material were of low variability and allowed for assessment of seasonal nutrient trends and comparison of values between different *Protea* varieties.

Materials and methods

Study sites and plant management

This study was conducted at 3 commercial plantations in the Mt Lofty Ranges, South Australia (35°10'S, 138°34'E) over 3 years, 1991–93. The climate is mediterranean, with cool, wet winters and dry, warm summers. The 3 sites differed in soil type which ranged from sandy loam over clay (site 1) to highly leached sands (site 3). Selected chemical and physical properties of the soil at each site are presented in Table 1.

From each site, 6 vigorously growing 5–7-year-old plants were selected for plant growth and yield monitoring commencing in June 1990. Plants of this age are in full production and their size is between 1 and 1.5 m wide and 1.5 and 2 m tall. Plants remained under the management of the various growers for irrigation, fertiliser and pest management. Current standard cultivation practices include minimal fertiliser programs, such as sidedressing in spring with nitrogen (N) and micronutrient applications when needed. Supplemental drip irrigation at 8–12 L/plant, was generally applied 1–2 times/week from October to June, and pruning either coinciding with or soon after harvest, to shape plants and remove damaged and diseased material.

Experimental details

At each site, 6 vegetative shoots were selected during March (Pink Ice) and July (Silvan Red) on each of the 6 plants to be monitored for growth during the following year. Young shoots which had developed during the previous summer (current season's growth only) were selected and tagged. Monthly measurements of shoot elongation and shoot diameter (at 20 cm above the base of the shoot) were made. Growth was monitored until the shoot was harvested as a flowering stem or pruned due to loss of the growing tip. If the flower bud aborted, lateral shoots commenced development and measurement of shoot elongation ceased.

All material removed from the trial plants by harvest or pruning was recorded. During the harvest period of Pink Ice (March–November), recordings were made at 3–14-day intervals, depending on the time of harvest chosen by the growers. Silvan Red was harvested 1–3 times/year at each site, when the grower deemed the market to be most favorable. This cultivar is most frequently harvested in March as a red-foliaged stem, however, it can remain on the bush for a secondary harvest in June–July when it presents as a tricolor stem—yellow, red and green. Flowers were cut leaving a stub of about 10 cm and side shoots were removed. Each flowering stem was measured from the cut end to the tip of the flower head (Pink Ice) or involucral leaves (Silvan Red) and all stems and prunings were weighed fresh. Pruning was also carried out during harvesting and at the completion of harvest to eliminate dead buds, distorted and misplaced shoots and to shape the plant.

Table 1. Selected chemical and physical properties of soil at three sampling sites in South Australia

Soil depth (cm)	pH[A]	Organic C (%)	Extractable P[B] (mg/kg)	Extractable K[B] (mg/kg)	Particle size analysis (%) Sand	Silt	Clay
			Site 1				
0–15	4.8	2.7	15	113	85	10	4
15–70	5.3	5.6	—	—	—	—	—
			Site 2				
0–20	7.0	0.4	14	43	95	2	2
20–70	6.8	0.1	9	48	—	—	—
			Site 3				
0–15	6.2	1.4	64	92	98	1	1
15–80	6.1	0.3	13	37	—	—	—

[A] 1 : 5, soil : water ratio.
[B] 1 : 100, soil : 0.5 mmol/L $NaHCO_3$, 16 h shaking time.

Statistical analysis

For each site, the significance of the differences in yield between years was determined by analysis of variance. The 6 plants monitored at each site were considered replicates (n = 6). Least significant differences at P = 0.05, were used to separate means. For monthly extension growth and stem diameter, and monthly cumulative yields for Pink Ice, s.e.m. values were calculated.

Results and discussion

Soil chemical and physical properties

Soil acidity varied from neutral throughout the profile at site 2 to strongly acid at site 1 (Table 1). However, even though the soil at site 1 was strongly acid, annual yields of Pink Ice and Silvan Red were not reduced compared with sites 2 and 3 (Tables 2 and 3). These data suggest that these 2 hybrids are tolerant of high soil acidity.

Extractable phosphorus (P) concentrations in the 0–15 and 0–20 cm surface soils ranged from 64 mg/kg at site 3 to 14 and 15 mg/kg at sites 2 and 1 respectively. However, this high residual P concentration was not associated with reduced yields (Cecil *et al.* 1995; Maier *et al.* 1995). The potassium (K) requirement of proteas for optimum growth appears to be low (Maier *et al.* 1995) and we suggest that the critical extractable-K concentration for soil types at these sites is low (<50 mg/kg) and the sites were not deficient in K. At our sites, extractable-K concentrations in the surface soils ranged from 43 to 113 mg/kg and were not K deficient.

On the basis of organic carbon (C), soil at site 1 was classified as highly fertile, site 2 was infertile, and site 3, moderately fertile with regard to N (Maier *et al.* 1995). These data suggest that higher rates of N fertiliser may be required at site 2 compared with site 1. However, appropriate nutrient management technology, such as calibrated plant or soil tests, are not currently available for growers to use to determine the N fertiliser requirement of their plantings.

Yield

Table 2 presents yield of Pink Ice in terms of fresh weight and stem numbers at 3 sites over 3 harvests, 1991–93. At sites 1 and 2, there are no significant differences in total biomass removed between years, however, at site 3, yields of biomass, prunings and stems were significantly higher in the 1993 season. At site 1, the weight removed in prunings doubled between the first and second year, which may have influenced the number and weight of stems in the subsequent year.

Yield (stems/plant) is presented by size categories reflecting marketing grades used by the industry in Australia for domestic and export quality flowers. There is a relatively consistent spread of stems in all size classifications throughout the different sites in different years. Site 1, the highest yielding site in terms of both total stem numbers and biomass, also had the highest number of stems in the <50 and >90 cm size classes. There were significant differences in total stem numbers between years at sites 1 and 3, but not at site 2, the lowest yielding site. These yearly differences may be a reflection of plant age, pruning practices or a response to the higher than average rainfall experienced in the winter of 1992.

Analysis of between-site differences showed that weight (biomass) and number of flowering stems were

Table 2. Yield of *Protea* 'Pink Ice' at three commercial sites in South Australia, 1991–93

Means within columns and sites, followed by the same letter are not significantly different at P = 0.05

Year	Yield (kg/plant)			Yield (stems/plant)				
	Biomass	Prunings	Stems	<50 cm	50–70 cm	70–90 cm	>90 cm	Total
				Site 1				
1991	16.5a	4.6a	11.9a	2a	27a	26a	1a	58a
1992	21.1a	9.6b	11.5a	4a	24a	22a	5b	55a
1993	21.7a	6.9b	14.7b	11b	34a	22a	8b	76b
	n.s.	*	*	*	n.s.	n.s.	*	*
				Site 2				
1991	12.5a	3.8a	8.8a	2a	18a	15a	3a	40a
1992	12.5a	5.3b	7.3a	5a	19a	13a	1b	37a
1993	14.1a	6.1b	8.1a	5a	19a	13a	3a	39a
	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.
				Site 3				
1991	14.7a	5.1a	9.5a	1a	18a	24a	2a	44a
1992	16.1a	5.6a	10.5a	1a	24a	24a	3a	52b
1993	22.4b	8.0b	14.4b	8b	35b	26a	6a	74c
	***	**	***	**	**	n.s.	n.s.	***

* P<0.05; ** P<0.01; *** P<0.001; n.s., not significant.

Table 3. Yield of *Leucadendron* 'Silvan Red' at three commercial sites in South Australia, 1991–93
Means within columns and sites, followed by the same letter are not significantly different at $P = 0.05$

Year	Yield (kg/plant)			Yield (stems/plant)			
	Biomass	Prunings	Stems	40–60 cm	60–80 cm	>80 cm	Total
			Site 1				
1991	10.6a	1.7a	8.9a	130a	106a	53a	289a
1992	16.6b	4.2b	12.3b	163a	95a	72a	330a
1993	14.4c	2.9c	11.2b	125a	131b	68a	324a
	***	***	**	n.s.	*	n.s.	n.s.
			Site 2				
1991	10.5a	3.6a	6.9a	121a	63a	38a	222a
1992	10.8a	4.4a	6.4a	109a	65a	35a	209a
1993	8.3a	1.1b	7.4a	98a	67a	61b	226a
	n.s.	***	n.s.	n.s.	n.s.	*	n.s.
			Site 3				
1991	9.5a	2.0a	6.1a	138a	82a	9a	229a
1992	15.1b	3.3b	11.8b	139a	96a	64b	298a
	*	*	*	n.s.	n.s.	**	n.s.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; n.s., not significant.

lower at site 2 compared with sites 1 and 3. Average yield (± s.e.) of stems over 3 years at site 1 was 63 (± 7) stems/plant, 39 (± 1) stems/plant at site 2 and 57 (± 9) stems/plant at site 3.

Differences in yields between sites may be a reflection of the N status of plants grown in different soil types. Leaf N concentrations were also consistently lower throughout the year at site 2 compared with sites 1 and 3. During the active growth stage in spring, leaf N values at site 2 were in the range 0.8–0.9%, while those at the other sites were in the range 1.1–1.2% (Maier *et al.* 1995).

Cumulative yield of Pink Ice stems harvested per plant during the total harvest period, March–November, is presented in Figure 1. At site 1, with highest soil fertility (Table 1), flower maturation extended over a longer period than at the other sites, with a subsequent 8-month harvest period. At site 3, cumulative yield reached similar levels, however, harvest was more concentrated in April–June, where it then continued at low levels and ceased in September. At site 2, the poorest yielding site, 85% of flowers were harvested by the end of a 2-month period (Fig. 1).

These results are of significance in that they show that under similar climatic conditions, length of harvest of Pink Ice can be influenced by soil fertility levels, and by inference, nutritional management of the crop. It is generally agreed that it is advantageous for producers to have an extended harvest period with fresh flower crops as it allows for continuity of supply to florists and export markets, and therefore greater maintenance of product identity and market share. In addition, there are greater efficiencies in management and utilisation of labour. We suggest that a restricted flower maturation period (6 weeks–3 months) for this variety is an indication of nutritional or other growth stress that should be addressed for increased productivity.

Yield of Silvan Red in terms of weight of prunings, stems and total biomass varied significantly between years at sites 1 and 3 and only in weight of prunings at site 2 (Table 3). However, yield differences in total stem numbers were not significant between years. Although

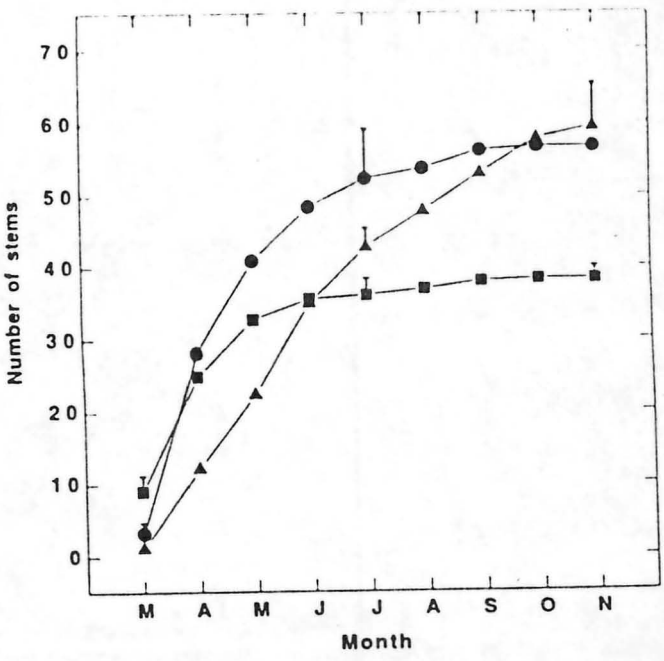

Figure 1. Cumulative yield of flowering stems of *Protea* 'Pink Ice' at sites 1 (▲), 2 (■) and 3 (●) in the Mt Lofty Ranges, South Australia, averaged over 3 years. Vertical bars represent +1 s.e.

yield of this cultivar at site 2 was consistently lower than at the other sites, analysis of between-site comparisons does not show significant differences in either biomass or number of stems until the third year. Total stems harvested over the 3-year study averaged (± s.e.) 314 (± 13) stems/plant at site 1, 219 (± 5) stems/plant at site 2, and 264 (± 35) stems/plant at site 3.

The yield data presented here can be used in comparative terms to assess the productivity of different commercial plantings within a region or between different regions (countries). They also serve as a basis of economic analyses and can be useful in feasibility studies and monitoring enterprise performance (Kernick *et al.* 1989). We suggest that a productive commercial planting of Pink Ice should annually yield between 50 and 65 stems/plant with a stem length greater than 50 cm and Silvan Red should yield between 250 and 300 stems/plant.

This yield data can also be used to develop maintenance fertiliser programs for commercial growers. A calibrated plant or soil test cannot predict the amount of fertiliser required when low concentrations or deficiencies are diagnosed. The 'balance sheet' approach is one way to determine amounts of nutrients to apply (Sparrow 1993). Based on accurate yield and chemical composition data, the amounts of nutrients removed in produce can be determined and these values used as a basis of a nutrient replacement program. Maier *et al.* (1995) showed that between 14–20 g N, 7–9 g K, 19–28 g calcium (Ca) and 2–3 g magnesium (Mg) were removed per plant during harvest of Pink Ice and similarly, 15–19 g N, 7–10 g K, 17–19 g Ca and 8–11 g Mg were removed per plant by Silvan Red (Cecil *et al.* 1995). Based on these values we would recommend an annual nutrient replacement application of Ca and N up to 20–30 g/plant, K up to 10–15 g/plant and Mg up to 5–10 g/plant for both cultivars.

Seasonal growth flushing

Figure 2 presents results of monthly growth measurements of stem elongation of the 2 cultivars averaged over 3 years at 3 separate sites. With both cultivars there are marked seasonal patterns in stem growth. Stem length growth curves follow a similar trend for all 3 sites, with up to 1 month later growth patterns evident with both varieties at site 1, located in the coolest growing district.

Protea *Pink Ice.* Stem elongation commenced in early spring, reaching its peak at sites 2 and 3 in October (Fig. 2*a*). Growth elongation was delayed at site 1 with a more dramatic increase in November. By December, elongation at all sites was at a similar low level with a small secondary growth flush in February at site 1. After April, elongation growth ceased at all sites until August. During the period of peak growth activity, stem elongation at all sites averaged 114 mm in a 1 month period.

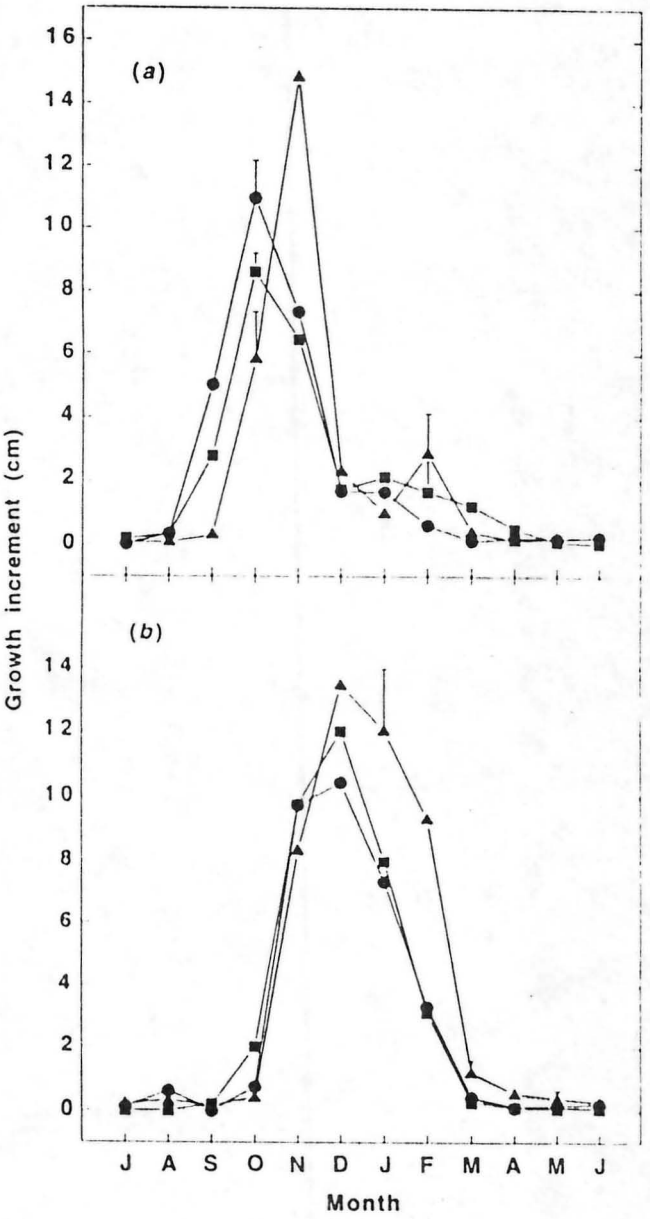

Figure 2. Mean monthly stem elongation (cm) of (*a*) *Protea* 'Pink Ice' and (*b*) *Leucadendron* 'Silvan Red' at sites 1 (▲), 2 (■) and 3 (●) in the Mt Lofty Ranges, South Australia, averaged over 3 years. Vertical bars represent +1 s.e.

This pattern of seasonal stem growth in Pink Ice is analogous to that of *P. neriifolia* Kouga reported in South Africa (Heinsohn and Pammenter 1988) where extension growth of reproductive shoots reached a peak in September (mean of 80 mm) with minimal secondary flushing of less than 4 mm in January. There was no stem elongation between March and August. Dupee and Goodwin (1992) reported peak growth of vegetative and reproductive shoots of *P. neriifolia* Salmon Pink grown in New South Wales to be in the period January–February.

Leucadendron *Silvan Red.* Growth commenced at all

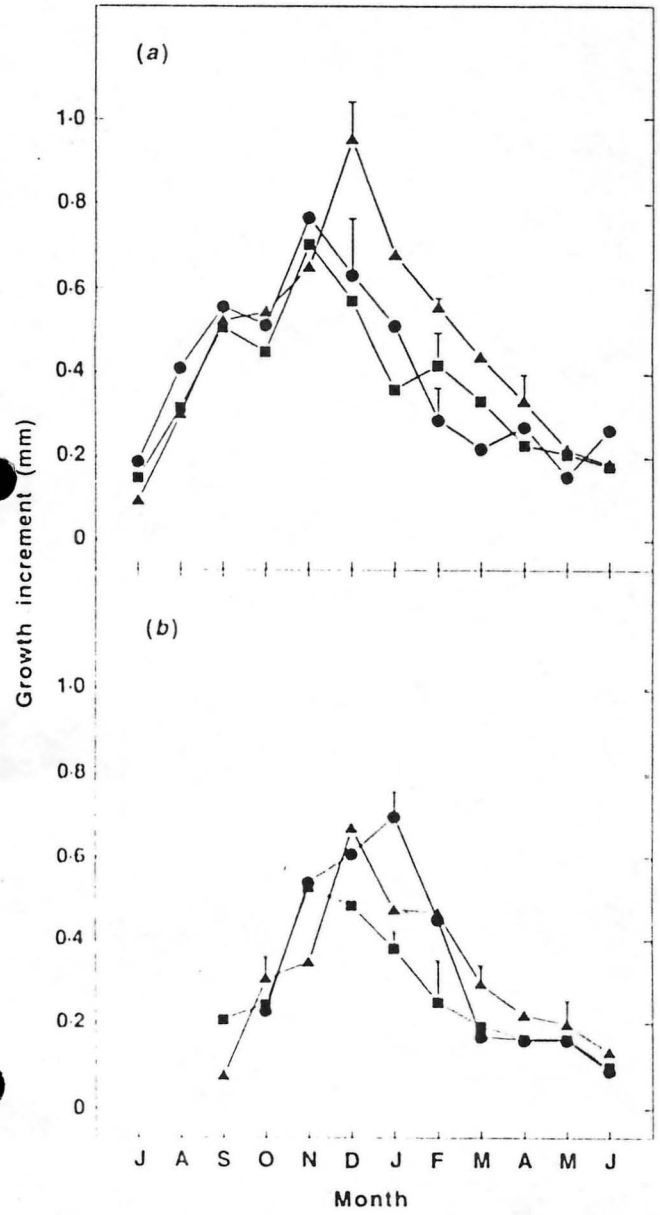

Figure 3. Mean monthly increase in stem diameter (mm) of (*a*) *Protea* 'Pink Ice' and (*b*) *Leucadendron* 'Silvan Red' at sites 1 (▲), 2 (■) and 3 (●) in the Mt Lofty Ranges, South Australia, averaged over 3 years. Vertical bars represent +1 s.e.

sites between October and November and ceased by March. Stem elongation activity was sustained at a higher level at site 1 through summer than at the other 2 sites where growth peaked in December. This response is analogous to the growth trend at this site with Pink Ice, however, elongation occurs longer into the summer months. There was minimal stem elongation at all sites from autumn until spring. During the period of peak growth activity, stem elongation at all sites averaged 119 mm in a 1 month period.

Figure 3 presents monthly growth measurements of stem diameter averaged over 3 years for each of the 3 sites. In both cultivars, maximum increase in stem diameter occurs at the same time as maximum stem elongation, however, stem diameter growth is more sustained throughout the year. During the period of peak growth activity, the average increase in stem diameter of Pink Ice was 0.81 mm/month and Silvan Red 0.63 mm/month.

Stephenson *et al.* (1986) and George *et al.* (1989) have highlighted the effect of vegetative flushing on leaf nutrient concentrations and concluded that an understanding of the timing and extent of flushing is important to ensure correct interpretation of plant test data. The flushing patterns we have presented can be used to define leaf sampling times for Pink Ice and Silvan Red on a phenological basis rather than chronologically (Cecil *et al.* 1995; Maier *et al.* 1995). This means that plant test data collected from different climatic conditions or from plants grown under different management practices have common reference growth stages for more accurate comparison to standards that have been defined by the growth response of these varieties.

References

Barth, G. E. (1994). Growth, yield and nutrient monitoring of *Protea* 'Pink Ice' and *Leucadendron* 'Silvan Red'. *In* 'Proceedings of the Third National Workshop for Australian Native Flowers'. University of Queensland, Gatton. (Ed. Story Horticultural Services.) pp. 7-22–7.

Brits, G. J. (1992). Breeding programmes for Proteaceae cultivar development. *Acta Horticulturae* **316**, 9–17.

Cecil, J. S., Barth, G. E., Maier N. A., Chvyl, W. L., and Bartetzko, M. N. (1995). Leaf chemical composition and nutrient removal by stems of *Leucadendron* cvv. Silvan Red and Safari Sunset. *Australian Journal of Experimental Agriculture* **35**, 547–55.

Dupee, S. A., and Goodwin, P. B. (1992). Flowering and vegetative growth of *Protea neriifolia* and *Protea cynaroides*. *Acta Horticulturae* **316**, 81–97.

Fuss, A. M., and Sedgley, M. (1991). Variability in cut flower production of *Banksia coccinea* R. Br. and *Banksia menziesii* R. Br. at six locations in southern Australia. *Australian Journal of Experimental Agriculture* **31**, 853–8.

George, A. P., Nissen, R. J., and Carseldine, M. L. (1989). Effect of season (vegetative flushing) and leaf position on the leaf nutrient composition of *Annona* spp. hybrid cv. Pink's Mammoth in south-eastern Queensland. *Australian Journal of Experimental Agriculture* **29**, 587–95.

Heinsohn, R. D., and Pammenter, N. W. (1988). Seasonality of shoot growth and flowering in the fynbos shrub *Protea neriifolia* cultivated in a summer rainfall area. *South African Journal of Botany* **54**, 440–4.

Kernick, C., Barth, G. E., and Ronan, G. (1989). The economics of carnation and chrysanthemum growing. South Australian Department of Agriculture Market Development Paper No. 8. Agdex 280/821, Adelaide. 32 pp.

Maier, N. A., Barth, G. E., Bartetzko, M. N., Cecil, J. S., and Chvyl, W. L. (1995). Effect of sampling time and leaf position on leaf nutrient composition of *Protea* 'Pink Ice'. *Australian Journal of Experimental Agriculture* **35**, 275–83.

Sparrow, L. A. (1993). A review of fertiliser advice in Australia. *Australian Journal of Experimental Agriculture* **33**, 1067–77.

Stephenson, R. A., Cull, B. W., Mayer, D. G., Price, G., and Stock, J. (1986). Seasonal patterns of macadamia leaf nutrient levels in south east Queensland. *Scientia Horticulturae* **30**, 63–71.

Wallerstein, I., and Nissim, A. (1992). Control of growth and flowering in *Banksia ashbyi, Leucospermum patersonii* and *Leucadendron* 'Safari Sunset'. *Acta Horticulturae* **316**, 73–80.

Yencken, J. (1994). The Australian Flower Industry: a review. Rural Industries Research and Development Corporation. Research Paper No. 94/9, Barton, ACT. 301 pp.

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SO: Journal-of-the-Southern-African-Society-for-Horticultural-Sciences. 1994, 4: 1, 42-46; 5 ref.
DE: pruning-; seasons-; flowers-; development-; cut-flowers; production-; cultural-methods; ornamental-plants; ornamental-woody-plants

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KEYWORDS: Flowering, *Proteaceae*, pruning.

ABSTRACT

Plants of *Protea* cv. Carnival were pruned at different times, viz. 12 March, 9 April, 21 May, 2 July, 13 August and 17 September 1991 (Southern hemisphere). Plants pruned on 2 July 1991 and later did not flower the following season. Pruning on the first three dates produced inflorescences in a decreasing number when pruning was delayed from 12 March 1991 to 21 May 1991. Apparently, at least two consecutive flushes are needed for inflorescences to form, with no inflorescences forming on the autumn or second summer flushes. Varying the number of bearers per trunk circumference on both two- and six-year-old plants produced no significant differences in yield. Current pruning practices, where plants are pruned during harvest from February to May, result in a low yield of saleable flowers of 'Carnival'. Pruning manipulations, such as varying the number of bearers and delaying the pruning time, did not increase the yield in the following season.

INTRODUCTION

Protea cv. Carnival (a natural hybrid, probably of *P. compacta* and *P. neriifolia*) is grown commercially in the Stellenbosch district in South Africa. Cut flowers are exported to Europe.

'Carnival' flowers are borne terminally on the current season's shoots and are picked from February to May (Southern hemisphere). Short stumps (bearers) of about 15 cm long are left when flowers are picked. New growth for the following year's crop sprouts from axillary buds on the bearers. After harvest, non-flowering shoots longer than 35 cm are also headed back to leave a bearer, and the remaining shoots are removed at their base with a thinning cut. Yields are, however, disappointingly low and flower stems are short, seldom reaching a desirable length of 50 cm. In this paper, we report on the effect of the time of pruning and the number of bearers left per plant on the yield of saleable flowers (straight stems longer than 35 cm) produced on *Protea* cv. Carnival.

MATERIALS AND METHODS

Plant material

Six-year-old clonal plants of *Protea* cv. Carnival from a commercial plantation were used. They were grown under natural climatic conditions near Stellenbosch in the Western Cape, South Africa (33°54'S). The plants, spaced 1 m apart with 4 m between rows, were not irrigated or fertilized. The mean annual rainfall is 600-700 mm (Schulze, 1974) and occurs mainly during winter (July in the Southern hemisphere). The longest day is 14.25 hr and the shortest day 9.53 hr long.

Pruning date

The current season's flowering and non-flowering shoots longer than 35 cm were headed back to just below the intercalation between the first and second growth flushes on the shoot. The remaining stump, hereafter referred to as a bearer, varied in length from 11-19 cm. The trunk circumference of each plant was measured 10 cm above ground level, whereafter the number of bearers per plant were reduced to 2.5 bearers per cm trunk circumference with a thinning cut at the point of inception. The time of pruning was varied, viz. early (12 March, 9 April and 21 May), and late (2 July, 13 August and 17 September 1991). Spring growth started just before the last date.

After pruning, a branch with a number of bearers was tagged on each plant. Data were collected on the bearers of the tagged branch units until 24 April 1992. The number of shoots, the time of sprouting, the shoot length of each growth flush, and the number of flushes before inflorescence formation were recorded.

At the commercial harvest stage, flowering shoots were picked with a thinning cut and brought to our laboratory. This was

TABLE 1: The effect of the time of pruning on the total fresh weight and fresh weight of flowering shoots of *Protea* cv. Carnival.

	Fresh weight (kg/plant)		
Pruning date	Total	Flowering shoots	% Reproductive
Early:			
12 Mar 1991	7.7	3.1	43.4
09 Apr 1991	4.8	1.8	32.9
21 May 1991	3.6	0.6	17.7
Late:			
02 July 1991	-	0.0	0.0
13 Aug 1991	-	0.0	0.0
17 Sept 1991	-	0.0	0.0
Source [z]			
Early $_{\text{Linear}}$	0.0001	0.0003	0.0023
Early $_{\text{Quadratic}}$	0.8079	0.4721	0.2514

[z] Probability of a significant F-value.

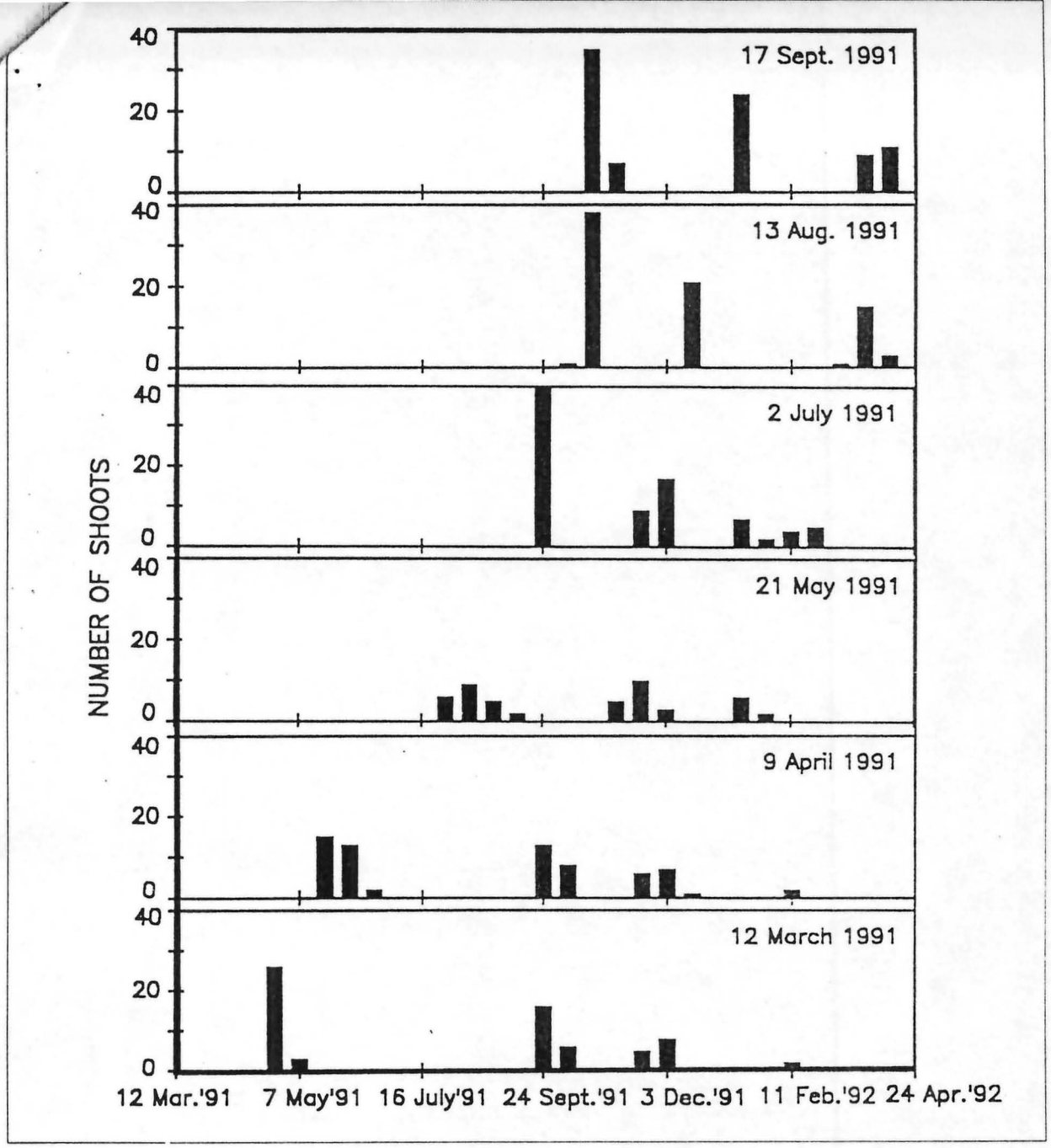

FIGURE 1: The effect of the time of pruning on the seasonal growth flushes of *Protea* cv. Carnival.

repeated three times a week until the entire crop had been harvested. The length of the stems, the mass of the flower and the stem, and the number of saleable flowers (straight stems longer than 35 cm) were recorded. On 24 April 1992, all non-flowering shoots of plants pruned on the first three dates were cut off. Shoots were classified as longer than or shorter than 35 cm, whereafter they were counted and weighed.

Single plants were used per treatment. Treatments were replicated ten times in a randomized complete block design.

Number of bearers

Two- and six-year-old, clonal plants of 'Carnival' were pruned on 12 March 1991 by the same method as described above. The number of bearers left per plant varied: on two-year-old plants 1.0, 1.5, 2.0, and 2.5, and on six-year-old plants 1.5, 2.0, 2.5, 3.0, and 3.5 bearers were left per cm trunk circumference. Flowers were picked as described earlier. The number of flowers picked was expressed per plant, per bearer, or per cm trunk circumference.

In both age groups, one plant was used per treatment. Treatments were repeated five times in a randomized complete block design.

Data analysis

The General Linear Means (GLM) procedure in the Statistical Analysis System

TABLE 2: The effect of the time of pruning on the production of flowering and non-flowering shoots of *Protea* cv. Carnival.

Pruning date	Number of stems per plant			
	Flower flower	Saleable > 35cm	Non-flowering	Saleable (%)
Early:				
12 Mar 1991	19.9	5.9	6.3	29.6
09 Apr 1991	11.8	4.4	11.9	37.3
21 May 1991	6.0	3.0	17.3	50.0
Late:				
02 July 1991	0.0	0.0	-	-
13 Aug 1991	0.0	0.0	-	-
17 Sept 1991	0.0	0.0	-	-
Source [z]				
Early $_{Linear}$	0.0004	0.1277	0.0007	
Early $_{Quadratic}$	0.6499	0.7754	0.4150	

[z] Probability for a significant F-value.

TABLE 3: The effect of the time of pruning on flower position on shoots of *Protea* cv. Carnival.

Pruning date	Percentage flowers			
	Autumn flush	Spring flush	1st summer flush	2nd summer flush
Early:				
12 Mar 1991	0.0	56.1	43.9	0.0
09 Apr 1991	0.0	52.7	47.3	0.0
21 May 1991	-	3.9	96.1	0.0
Late:				
02 July 1991	-	0.0	0.0	0.0
13 Aug 1991	-	0.0	0.0	0.0
17 Sept 1991	-	0.0	0.0	0.0
Source [z]				
Early $_{Linear}$		0.0030	0.0030	
Early $_{Quadratic}$		0.0159	0.0159	

[z] Probability of a significant F-value.

(SAS) programme was used to analyse the data (SAS Institute Inc.,1990).

RESULTS AND DISCUSSION

Pruning date

Plants pruned on 2 July 1991 or later did not flower the following season (Table 1 and 2). The total fresh weight of new shoot growth and flowers decreased from 7.7 to 3.6 kg per plant when the pruning date was delayed from 12 March 1991 to 21 May 1991 (Table 1). The fresh weight yield of both the non-flowering and flowering shoots decreased (Table 1). The latter was reduced more since the fresh weight yield of flowering shoots, expressed as a percentage of the total fresh weight, decreased from 43.4 to 17.7 (Table 1). This decrease was also reflected in the number of flowering shoots per plant, which was reduced from 19.9 to 6.0 (Table 2). When pruning was delayed till 21 May 1991, the number of saleable flowers per plant decreased from 5.9 to 3.0, and the number of shoots longer than 35 cm that failed to flower increased from 6.3 to 17.3 (Table 2). Therefore, the later pruning takes place, the smaller the probability that flowering shoots will develop.

Shoot growth of 'Carnival' occurs in flushes (Fig. 1). Variation in the time the flush started depended on the pruning date. Plants pruned on 12 March 1991 and 9 April 1991 flushed in April and May respectively (autumn flush). Bud swell, but no bud break, occurred on plants pruned on 21 May 1991. A spring flush originated either terminally on the autumn flush of plants pruned on 12 March 1991 and 9 April 1991, or from axillary buds on the bearers of plants pruned 21 May 1991 or later. The spring flush sprouting from swollen buds on plants pruned on 21 May 1991 started about one month earlier than the spring flush on plants pruned on 12 March 1991 and 9 April 1991. The spring flush on plants pruned on 13 August 1991 or 17 September 1991 sprouted about one month later than the spring flush of plants pruned on 12 March 1991 and 9 April 1991. A summer flush occurred from early to late December, followed by a second summer flush from February to April.

Low winter temperatures may be partially responsible for plants not sprouting immediately following winter pruning. The mean temperatures for May, June, July and August are 14.1, 13.1, 11.4 and 11.7C respectively (Matthee, 1982). Short days in winter cause non-sprouting in other members of the Proteaceae and may also be implicated in 'Carnival' (Malan & Jacobs, 1990; Malan & Brits, 1990). When natural day lengths were extended with low intensity incandescent lighting throughout the night, shoot growth occurred in *Leucospermum* cv. Red Sunset (Malan & Jacobs, 1990), *Serruria florida* (Malan & Brits, 1990) and *Protea* cv. Ivy (Jacobs, unpublished data).

Inflorescences did not develop on the autumn or the second summer flush (Table 3). Inflorescences formed in more or less even proportions on either the spring or the first summer flush of plants pruned on 12 March 1991 or 9 April 1991. Plants pruned on 21 May 1991 flowered mainly on the first summer flush (Table 3). Apparently, at least two consecutive flushes of shoot growth were necessary for flower formation. These consisted of either the autumn (April/May) and the spring (August/-September) flush for plants pruned on 12 March 1991 or 9 April 1991, or the spring and first summer flush for plants pruned on 21 May 1991 (Table 3). Limited leaf area and carbohydrates may be the reason inflorescences did not form on the autumn flush. Reasons for inflorescences failing to form on the second summer flush are not known.

Low yields of 'Carnival' are not related to a shortage of shoots; in fact there is an increase in the number of spring, first sum-

mer and second summer shoots on the plants that did not flower (last three dates) compared to those that did flower (first three dates). Low yields are therefore related to the date of pruning and not to the number of shoots produced on the bearer (Table 4).

The flush on which a flower initiated determined the length of the flower stem. Flower initiation on the spring flush invariably lead to short flower stems, whereas longer stems are evident when flower initiation occurred on the first summer flush (Table 5).

Number of bearers

Varying the number of bearers per cm trunk circumference from 1.5 to 3.5 for six-year-old plants and from 1.0 to 2.5 for two-year-old plants, did not increase the number of flowers per plant or per cm trunk circumference. The number of flowers per bearer decreased with an increase in the number of bearers per cm trunk circumference. The decrease was significant in the six-year-old plants, but not in the two-year-old plants (Table 6).

It is clear that current pruning practices, where plants are pruned during harvest from February to May, result in a low yield of saleable flowers of 'Carnival'. Pruning manipulations, such as varying the number of bearers and delaying the pruning time, did not increase the yield in the following season. Alternative pruning strategies should therefore be considered. This is the aim of another study, which is in progress.

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REFERENCES

MALAN, G. & JACOBS, G., 1990. Effect of photoperiod and shoot decapitation on flowering of *Leucospermum* 'Red Sunset'. *J. Amer. Soc. Hort. Sci.* **115**:131-135.

MALAN, D.G. & BRITS, G.J., 1990. Flower structure and the influence of day length on flower initiation of *Serruria florida* Knight (Proteaceae). *Acta. Hort.* **264**:87-92.

MATTHEE, G.W., 1982. Koue-eenhede in die winterreëngebied van Suid-Afrika. Afdeling Landbou en Inligting. Pretoria.

SAS INSTITUTE Inc., 1990. SAS/STAT User's Guide, Version 6, 4th ed, Vol. 1 and 2. Cary, NC.

SCHULZE, B.R., 1974. Climate of South Africa. Part 8. General survey. Weather Bureau. Department of Transport. Pretoria.

TABLE 4. The effect of the time of pruning on the number of shoots per bearer of *Protea* cv. Carnival.

	Number of shoots per bearer			
Pruning date	**Autumn flush**	**Spring flush**	**1st summer flush**	**2nd summer flush**
Early:				
12 Mar 1991	4.1	3.1	1.6	0.30
09 Apr 1991	3.6	2.8	1.8	0.61
21 May 1991	-	2.9	2.2	1.12
Late:				
02 July 1991	-	3.3	1.9	2.78
13 Aug 1991	-	3.6	2.5	2.84
17 Sept 1991	-	3.9	2.4	2.94
Source [z]				
Pruning date:				
Linear	0.0814	0.0004		
Quadratic		0.0819		
Early vs Late		0.0004		0.0001
Early Linear		0.0059		0.0035
Early Quadratic		0.8482		0.9367
Late Linear		0.0070		0.0052
Late Quadratic		0.4824		0.5628

[z] Probability of a significant F-value.

TABLE 5: The effect of the time of pruning on the mean shoot length of *Protea* cv. Carnival.

	Mean shoot length (cm)			
Pruning date	**Autumn flush**	**Spring flush**	**1st summer flush**	**2nd summer flush**
Early:				
12 Mar 1991	14.5	21.1	23.8	21.5
09 Apr 1991	10.8	18.7	22.3	20.7
21 May 1991	-	24.2	23.3	20.2
Late:				
02 July 1991	-	22.1	24.2	24.9
13 Aug 1991	-	22.0	24.5	28.4
17 Sept 1991	-	22.8	23.2	28.4
Source [z]				
Pruning date				
Linear	0.0029	0.0018		
Quadratic		0.0942		
Early vs Late			0.1516	0.0001
Early Linear			0.8154	0.7069
Early Quadratic			0.2055	0.9136
Late Linear			0.6684	0.7181
Late Quadratic			0.2201	0.9616

[z] Probability of a significant F-value.

TABLE 6: The effect of varying the density of bearers on the yield of six-year-old plants of *Protea* cv. Carnival.

No. of bearers per trunk circumference	Flowers/ bush	Flowers/ bearer	Flowers/cm trunk circumference
Two-year-old plants:			
1.0	14.1	1.28	1.31
1.5	15.4	0.98	1.43
2.0	11.3	0.59	1.04
2.5	16.3	0.74	1.45
Source [z]			
Number of bearers			
Linear	0.8594	0.1061	0.9826
Quadratic	0.4797	0.2971	0.5271
Six-year-old plants:			
1.5	17.6	0.58	0.88
2.0	18.5	0.46	0.91
2.5	12.8	0.28	0.68
3.0	15.9	0.23	0.73
3.5	22.2	0.31	1.08
Source [z]			
Number of bearers			
Linear	0.5102	0.0015	0.6244
Quadratic	0.1202	0.0403	0.1335

[z] Probability of a significant F-value.

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PREDICTING VASE LIFE IN TROPICAL CUT FLOWERS AND FOLIAGE

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Additional index words. ginger, heliconia, protea, anthurium, model.

Abstract. **Vase life of various tropical cut flowers and foliage was evaluated twice a week from harvest. Simple mathematical regression models were fitted to the vase life data. Loss of vase life varied with the different flowers. Linear models best described loss of quality in Banksia protea; fractional exponent models were best for gingers, lycopodium, bird-of-paradise and ti leaves, and bamboo orchid foliage; exponent models were best for anthuriums, and Sunset and pincushion protea; and logarithmic-fractional exponent models for heliconia. Validation tests verified the predictability of the vase life models for the heliconias, gingers, 'Midori' anthurium, lycopodium, and bamboo orchid foliage. The pattern of loss of flower quality provides the necessary basis to develop vase life criteria for different flowers and to schedule shipping by growers and marketing by wholesalers and retailers.**

Halevy and Mayak (1979) defined "vase life" as the useful longevity of the floral product at the final consumer's home. They also stated that the criterion for termination of vase life needs to be determined for each flower. Various points of termination of vase life have been utilized, from the sign of wilting (Mayak and Dilley, 1976) to 50% loss of flower quality (Paull et al., 1985) and total death of all flowers (Salinger, 1975). Previous studies on the vase life of cut flowers have examined post harvest physiology (Halevy and Mayak, 1981; Paull et al., 1985), and longevity of flowers harvested at different stages of maturity (Heuser and Evensen, 1986).

Many studies have measured the amount of time required for the flower to reach a particular stage of deterioration (Paull, 1982), but few have quantified the rate of aging or predicted vase life. Predictive models have been associated with quantifying floral changes. The long distances between suppliers and consumers make it desirable to know the rate of loss of vase life of different floral commodities. Simple mathematical models relating visual quality with time would allow a shipper to determine priority in shipping and a retailer to anticipate flower longevity and to manage inventory. The model would also serve as a research tool in comparing longevity of different cultivars.

The objectives of this study were: to determine if certain tropical cut flowers and foliage age at consistent, characteristic, commodity-specific rates; to develop simple mathematical models to describe aging in these commodities; and to validate these models by comparing the predicted state of aging at a specific time with the known state of another group of the same commodity at an equivalent time.

Materials and Methods

The post harvest life of a variety of popular tropical cut flowers and foliage was evaluated at the University of Hawaii at Manoa Beaumont Agricultural Research Center in Hilo, Hawaii. (Table 1). Most of the material was obtained fresh in the morning from a local commercial floral shipper before packing. Time of harvest varied with the grower. The protea were shipped from Maui and stored overnight before their initial evaluation. The cut flowers and foliage were maintained in an enclosed area under ambient conditions (ca. 25°C, ca. 50% R. H.). Vase water was changed weekly. No additives or preservatives were used.

The material was evaluated twice each week (3 to 4 days apart) by two observers each using a 10-point scale where

Table 1. Fitted regression equations and R^2 of models describing vase life of tropical cut flowers and foliage.

Commodity	Model	R^2
Anthurium (*Anthurium andraeanum* Lind.)		
'Ozaki'	$Y = -0.3 + 0.0136X^2$	0.945
'Midori'	$Y = 1.1 + 0.0012X^{2.5}$	0.964
Ginger (*Alpinia purpurata* [Vieill.] K. Schum.)		
Red	$Y = -1.6 + 2.2X^{0.7}$	0.855
Pink	$Y = -1.0 + 2.1X^{0.7}$	0.792
Heliconia (*Heliconia psittacorum* L. f.)		
'Andromeda'	$\ln Y = -4.4484 + 2.1531X^{0.4}$	0.994
'Parakeet'	$\ln Y = -4.7213 + 3.2525X^{0.3}$	0.937
'Parrot'	$\ln Y = -2.3872 + 2.8733X^{0.2}$	0.995
'Sassy'	$\ln Y = -3.7702 + 3.2161X^{0.3}$	0.969
Protea		
Pink Mink (*Protea neriifolia* R. B.)	$Y = -0.4 + 0.5X$	0.989
Orange banksi (*Banksia prionotes* Lindl.)	$Y = 0.1 + 0.6X$	0.957
Yellow-green banksia (*Banksia speciosa* R. Br.)	$Y = -0.8 + 0.6X$	0.939
Pink Frost (*Banksia menziesii* R. Br.)	$Y = 0.2 + 0.6X$	0.900
Safari Sunset (*Leucadendron salignum* Berg. × *L. laureolum* [Lam.] Fourcade)	$Y = 0.1 + 0.03X^2$	0.945
Scarlet Ribbon pincushion (*Leucospermum* spp. hybrid)	$Y = 0.6 + 0.02X^2$	0.964
Sunrise pincushion (*Leucospermum cordifolium* [Salisb. ex Knight] Fourcade)	$Y = 0.7 + 0.01X^2$	0.977
Hybrid 36 pincushion (*Leucospermum* spp. hybrid)	$Y = 0.3 + 0.2X^{1.5}$	0.985
Hybrid "A" pincushion (*Leucospermum* spp. hybrid)	$Y = 0.1 + 0.5X$	0.992
Foliage		
Lycopodium (*Lycopodium cernuum* L.)	$Y = -16.0 + 16.1X^{0.2}$	0.930
Ti (*Cordyline terminalis* [L.] Kunth)	$\ln Y = -3.9923 + 4.6753X^{0.1}$	0.982
Bamboo orchid (*Arundina graminifolia* [D. Don] Hochr.)	$\ln Y = -2.9824 + 3.8029X^{0.1}$	0.982
Bird-of-paradise (*Strelitzia reginae* Ait.)	$\ln Y = -12.7251 + 10.8364X^{0.1}$	0.956

0 = no damage, 2 = slight damage (10 to 20%) (limit of marketability), 4 = some obvious discoloration (limit of vase life), 7 = much discoloration throughout, and 9 = complete discoloration or death. The damage score for each flower or foliage was the sum of the two ratings. Hence, "0" was the minimum score and "18" was the maximum. The rating system has been used in other studies (Hansen et al., 1991, 1992).

Data used to develop the descriptive models usually were collected from five evaluated items per test. The 'Andromeda' heliconia and 'Parakeet' heliconia tests used ten items whereas the 'Scarlet Ribbon' and Hybrid "A" protea had four items each. Sample size for the gingers varied from three to six. The number of tests used in model development varied for each floral commodity. Bird-of-paradise and bamboo orchid foliage had two tests each; lycopodium, 'Parakeet' heliconia, and 'Ozaki' anthurium had three tests, pink ginger had four, and red ginger had thirteen. The remaining were tested once.

For each test, average ratings per observation were calculated by using the SAS Means and Regression Procedures (SAS Institute, 1982). Logarithmic and exponential transformation of both the independent term (day of observation) and dependent variable (average rating for that day) were examined. Criteria in determining the two-term regression model with the best fit were the largest coefficient of determination (R^2) and best graphic conformity with the earliest observations (those showing the smallest residuals). To examine model accuracy through shelf life, the average damage rating per observation was compared with the predicted value by using paired *t*-tests. Each had at least three observations. Protea and 'Parakeet' and 'Sassy' heliconia were unavailable for validation tests.

Results and Discussion

Aging for each floral commodity was characterized by a specific mathematical model (Table 1). In these model equations, "a" was the intercept parameter, "b" was the coefficient for the independent variable "*X*" which was expressed as time in *Days*, "n" was an exponent, and "*Y*" was the dependent variable which represented *Total Damage Rating*. The best model for heliconia was of the form: $\ln Y = a + bX^{0.n}$. The model described moderately long-lived commodities that initially deteriorate slowly, but for which degradation accelerates with time. The rate of aging accelerated the most with 'Sassy' heliconia, but was almost linear with 'Parrot' heliconia. Most foliage were best represented by a similar model, $\ln Y = a + bX^{0.1}$. These were long lasting materials whose rate of aging decreased with time. Ginger and lycopodium data produced a fractional exponential model, $Y = a + bX^{0.n}$. This represented a commodity that aged rapidly at first, then deteriorated slowly. The change in aging over time was slight for red ginger, but very pronounced for lycopodium. The anthuriums indicated an exponential model for aging, $Y = a + bX^n$. Flowers retained good visual quality for a long period of time, a desirable characteristic which allows latitude in the amount of shipping time, then rapidly deteriorated. Aging in Hybrid "A" and banksia protea was linear, $Y = a + bX$. Here, the rate of decay stayed constant. The remaining protea aged exponentially like the anthuriums. The protea were among the most long-lasting commodities evaluated; some can be used as dried flowers.

Assuming a total damage rating = 8 is the upper limit of visual acceptance, the models can be applied to determine average expected vase life of the floral commodities. The vase life of the heliconias (Fig. 1A) varied from a week ('Sassy') to 16 days ('Andromeda'). The vase life models for the foliage (Fig. 1B) indicated that ti leaves can last 2 weeks, and bamboo orchid foliage can last ca. 2.5 weeks. Lycopodium had the shortest vase life (a week). The models for the gingers indicated a vase life of about 8 days (Fig. 1C). The model for 'Midori' anthurium showed a vase life of over a month, while the model for 'Ozaki' anthurium indicated a vase life of 24 days (Fig. 1D).

The paired *t*-test showed no significant differences between predicted damage ratings and observed values from the validation tests for the heliconias, gingers, 'Midori' anthurium, bamboo orchid foliage, and lycopodium (Table 2). 'Ozaki' anthuriums that were significantly different had initial values that indicated some aging. The model for ti leaves was not verified.

The development of descriptive mathematical models requires certain conditions and assumptions. The floral material should have appropriate commercial quality so that the average aging in a sample will indicate a specific pattern found in the commodity. Production of accurate models that begin with no damage requires that the initial quality evaluations show very little aging. The models should be derived from the entire aging sequence up to death or complete discoloration. The model should predict the rate of aging with a precision that is independent of time of year, location, or source of propagation; potential site differences in aging are recognized, but are too minor to significantly alter the models. The models should ideally have no more than two terms for convenience and simplicity. Finally, post harvest conditions (e.g., vase water, temperature, humidity) of all commodities should be nearly equal.

Vase life models furnish useful information for floral marketing decisions. Because models are based from the time the commodity arrives at the packing house, they quantify the rate of aging that florists can use to anticipate vase

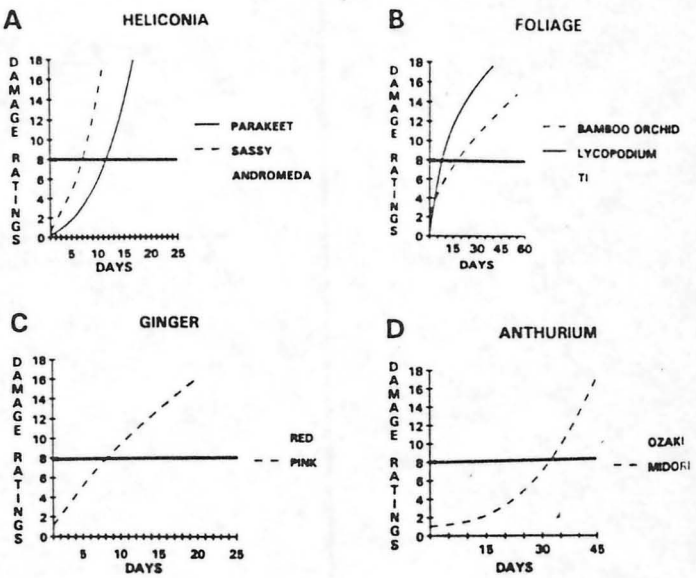

Fig. 1. Descriptive mathematical models of vase life for: A, heliconias; B, various foliage; C, gingers; and D, anthuriums. Bold line with arrow shows limit of damage rating for vase life.

Table 2. Summary of paired *t*-test comparing models describing vase life of tropical cut flowers and foliage with validation tests.

Commodity	Test	*n* Obs	*t*-value
Anthurium			
'Ozaki'	1	5	6.32**
'Ozaki'	2	4	5.27**
'Ozaki'	3	8	3.73**
'Ozaki'	4	8	2.09
'Midori'	1	9	1.61
Ginger			
Red	1	3	0.01
Red	2	3	2.14
Red	3	3	0.97
Pink	1	3	2.53
Heliconia			
'Andromeda'	1	5	2.42
'Parrot'	1	3	1.89
Foliage			
Lycopodium	1	3	1.57
Ti	1	4	4.64*
Bamboo Orchid	1	5	2.52
Bamboo Orchid	2	6	0.28

**, *t-test significant at $P = 0.01$ or 0.05, respectively.

life of their products. Hence, pricing structures of cut flowers and foliage can be arranged according to the expected vase life.

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Literature Cited

Halevy, A. H. and S. Mayak. 1979. Senescence and postharvest physiology of cut flowers: I. Hort. Rev. 1:204-236.

Halevy, A. H. and S. Mayak. 1981. Senescence and postharvest physiology of cut flowers: II. Hort. Rev. 3:59-143.

Hansen, J. D., H. T. Chan, Jr., A. H. Hara, and V. L. Tenbrink. 1991. Phytotoxic reaction of Hawaiian cut flowers and foliage to hydrogen cyanide fumigation. HortScience 26:53-56.

Hansen, J. D., A. H. Hara, and V. L. Tenbrink. 1992. Vapor heat: a potential treatment to disinfest tropical cut flowers and foliage. HortScience 27:(in press).

Heuser, C. W. and K. B. Evensen. 1986. Cut flower longevity of peony. J. Amer. Soc. Hort. Sci. 111:896-899.

Mayak, S. and A. Kofranek. 1976. Altering the sensitivity of carnation flowers (*Dianthus caryophyllus* L.) to ethylene. J. Amer. Soc. Hort. 101:503-506.

Paull, R. E. 1982. Anthurium (*Anthurium andraeanum* Andre) vase life evaluation criteria. HortScience 17:606-607.

Paull, R. E., N. J. Chen, and J. Deputy. 1985. Physiological changes associated with senescence of cut anthurium flowers. J. Amer. Soc. Hort. Sci. 110:156-162.

Salinger, J. P. 1975. Criteria for the evaluation of postharvest senescence of cut flowers. Acta Hort. 41:207-215.

SAS Institute. 1982. SAS User's Guide. SAS Institute, Cary, NC.

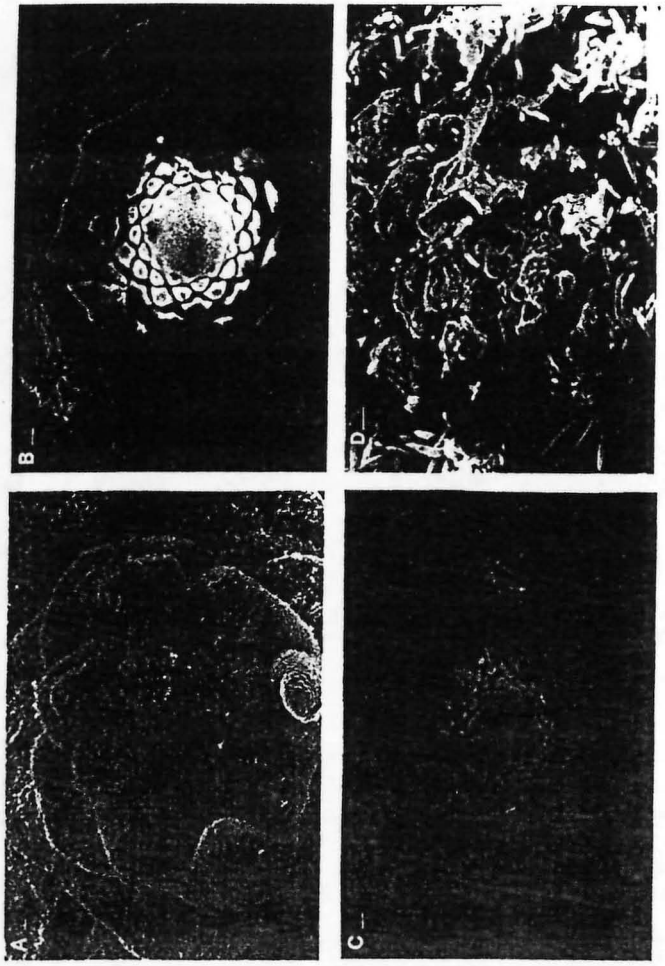

Fig. 4: Development of the terminal inflorescence of Serruria florida (SEM, bar = 100 µm). A. Apical meristem (on 3/2) initiating involucral bracts. B and C. Apical meristem (on 4/7 and 4/19 respectively) initiating bract and floret primordia. D. Inflorescence (on 5/4). Floret initiation completed. Perianth initials visible on florets. Hair-like structures on bracts and perianth obscure basal florets.

PROTEA POSTHARVEST BLACK LEAF A PROBLEM IN SEARCH OF A SOLUTION

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Abstract

It has been proposed that postharvest blackening of protea (*Protea neriifolia*) leaves is caused by water stress and/or insufficient carbohydrate supply. The following research was undertaken to determine the relative contribution of each to leaf blackening. The rate of development of leaf blackening varied with clonal source, season, and developed sooner in flowers harvested at the closed bud stage, with the slowest rate occurring in flowers harvested when the flower bracts had just started to unfold. Leaves were slower to blacken if harvested in the afternoon than in early morning. The immediate removal of field heat from flowers was only worthwhile if low temperature was maintained to the wholesale or retail level. High light intensities postharvest, solutions containing sugar, flower girdling and removal, significantly delayed the onset of leaf blackening. Water uptake by flowers rapidly declined after harvest with the commercial preservative solutions delaying the rate of decline in uptake. The decline in water uptake paralleled the loss in flower fresh weight. Preharvest irrigation regime did not significantly influence postharvest leaf blackening rate. The effect of flower carbohydrate supply on leaf blackening was felt to be modified by flower stem water uptake ability.

1. Introduction

Leaf blackening or browning of Protea flower, especially *Protea eximia* and *P. neriifolia*, is a serious problem postharvest (Ireland *et al.*, 1967). The leaf blackening leads to loss of decorative value, loss of market value and possible rejection of the consignment. A solution to this problem has not yet been achieved. Leaf removal is difficult without inflicting considerable damage to the stem because of the sessile nature of the leaves (Paull *et al.*, 1980).

The symptoms of this leaf disorder have been described many times (De Swardt *et al.*, 1987; Paull *et al.*, 1980; Brink and De Swardt, 1986; Ferreira, 1983; Haasbrock *et al.*, 1973). Leaf blackening have been divided into four types (De Swardt *et al.*, 1987) based upon the first appearance of the discoloration. The blackening probably occurs because of oxidation of phenols (Van Rheede van Oudtshoorn, 1963) and leuco-anthocyanins (Elsworth and Martin, 1971), that are found in protea leaves. The lack of relationship between these substrates, polyphenoloxidase and rate of leaf blackening (Ferreira, 1983) is not surprising

since the concentrations and activities are very high. The intensity and rate of symptoms development varies from year to year (De Swardt *et al.*, 1987), within a season and between different clones of one species (Akamine *et al.*, 1979; Paull *et al.* 1980).

Knowing what causes the blackening and regarding it as an abnormal senescence phenomenon have not enabled researchers to determine the trigger. Two major hypotheses are in vogue. One regards the leaf's water status as the trigger (Paull *et at.*, 1980; Ferreira, 1983; Mulder, 1977), with the other possible trigger being depletion of intracellular reserves possibly sugars (Brink and De Swardt, 1986). The relative importance of each may depend upon the actual circumstances.

Protea have been grown in Hawaii for some years (Parvin *et al.*, 1973). The postharvest handling system in Hawaii of protea places additional constraints on alternatives. Flowers are normally picked in the morning and packed either that day or within 24 hours in cardboard boxes. The boxes are all shipped by air without refrigeration. Hence, a solution in which refrigeration is not part of the handling system is desirable. The following work was undertaken to define the preharvest factors that lead to more rapid leaf blackening, then to attempt to reduce the rate of blackening by altering the rate of water loss and by providing sugars.

2. Material and Methods

Terminal shoots with flower buds of *Protea neriifolia* were either cut from several clones at the Maui Agricultural Research Center at Kula, Maui or obtained from a single commercial grower in the Kula area of Maui. The flowers were immediately boxed and air shipped to Honolulu where the investigations were carries out. The elapsed time from picking to initiation of an experiment was about 8 hours.

At least five stems were used for each treatment and all tests were repeated at least twice. Evaluation of the rate of leaf blackening was based upon the relative area of leaves with darkened surface as a percentage of total leaf surface area. Observation were recorded every two days until all the leaves were black. The time from harvest to 50% leaf blackening was used to calculate vase life. Observation on the center bracts of the flower were made for wilting and blackening. Vase life was evaluated under the following conditions: 20° to 25°C, 60 to 80% relative humidity, 10 hours fluorescent light (1 watt m^{-2} day^{-1}). All solutions were prepared in deionized water. Stems were recut following simulated shipping and prior to placement in solution.

3. Results and Discussion

3.1 Preharvest

Cultivar differences in rate of leaf blackening were reported for *Protea eximia* (Paull *et al.*, 1980), the differences being more pronounced when the flowers were held in commercial preservative solution. There was a three-fold

difference in the rate of leaf blackening between different clonal selections of *P. neriifolia* (Table 1). This clonal difference was not apparently associated with the different seasonal pattern of bush flowering. The Pink Splash clone peak flowering occurred between October through December, with Rose Mink in the November to January period and Pink Mink between July to November. Early season harvested flowers tended to be more prone to leaf blackening (Table 2). At the September-October period of the year the weather tended to be hotter and drier than later in the season.

Stage of flower opening at harvest significantly influenced days to 50% leaf blackening and days to moderate bract curling (Table 3). Leaves of flowers harvested in the closed bud stage blackened more rapidly than those harvested when the bracts were just unfolding or at a later stage. The optimum stage of harvest was when the bracts had just started to unfold as these flowers had longer total life than flowers picked at other stages.

Flowers harvested from part of a field of Pink Minks that had been irrigated at three week intervals did not show any significance in days to 50% leaf blackening and moderate bract curling from another part of the field receiving regular weekly irrigation. Flowers harvested in the early morning (8 am) had a shorter period to 50% leaf blackening than those harvested at 2 pm. Ferreira (1986) reporting the works of Muller (1977) indicates that an excessive loss of water was needed to initiate leaf browning. The above results do not fully support this conclusion.

3.2 Water Relations

The loss of water occurs both from the flower head and the leaves (Paull *et al.*, 1980). Water loss from the flower varied from 25 to 50% of the total. Removal of the flower head delayed leaf blackening (Table 4) as had been previously reported (Paull *et al.*, 1980). Girdling of stem just below the flower head also delayed leaf blackening to the same extent. This girdling result suggested that the effect of water loss from the head was not always the major factor initiating leaf blackening. The possibility of something being transferred from the flower head to leaves cannot be discounted.

Coating the leaves with various antitranspirants (Table 5) delayed leaf blackening though, the effect was not as marked, as the effect of flower head removal. The delay caused by antitranspirants was significant and repeatable.

Water uptake rate declined from ca. 150 ml day^{-1}, after recutting following 2 days packing, to 12 ml day^{-1} 6 days later. The rate of decline was less in flowers held in Florever (20 g l^{-1}), the uptake rate being 36 ml day^{-1} 6 days after recutting following 2 day pack. This agrees with our previously reported results (Paull *et al.*, 1980). De Swardt *et al.*, (1987) have suggested that a moisture loss of only 1% may be sufficient to initiate browning. It is unclear how this 1% loss relates to leaf water status. Akamine *et al.* (1979) only found a limited correlation between leaf dry weight and rate of leaf darkening, while Ferreira (1983) did find a significant relationship. Relative water content declines as leaf blackening increases (Figure 1). The relationship between

relative water content and leaf blackening did not answer the question as to whether water status was the trigger. Florever (20 g l^{-1}) delayed the decline in relative water content and rate of leaf blackening. These preliminary results have to be repeated.

3.3 Carbohydrate Relations

Use of preservatives in the vase solution reduced the rate of leaf browning, with sucrose being the most effective component (Akamine *et al.*, 1979; Brink and De Swardt, 1986). Exposure of flowers to high temperatures would stimulate respiration (Ferreira, 1986) and therefore deplete carbohydrate reserves, though water loss from these flower would be difficult to control. Pulsing of *P. neriifolia* flowers with greater than 7.5% sucrose was harmful and accelerated leaf browning. Brink and De Swardt (1986) indicated that a concentration higher than 1% was harmful yet a summary of Brink's PhD thesis (Brinks, 1987) stated 10%. The reason for the discrepancy is unclear. Our results support the 10% figure, however we were not able to greatly delay leaf blackening by pulsing with 10% w/v sucrose for 24 hours followed by 2 days pack.

Exposure of flower to high light had a significant effect in delaying leaf blackening (Table 6). Fluorescent light exposure (9 mE m^{-2} sec) was found to be inadequate in delaying leaf blackening. Increasing the period in simulated packing to 3 or more days significantly reduced the time to 50% leaf blackening (Table 7). These results suggest a significant role for light.

Brink's (1987) data is very useful in ascertaining where vase supplied carbohydrate end up in the cut flower. The results indicate that with longer periods (18 hours) of sucrose exposure, the flower head is the preferred sink (Brink and De Swardt, 1986). Leaves do receive significant amount of sucrose if the pulses are less than 12 hours (Brinks, 1986). He recommended a 12 hour pulse. Attempts to pulse flowers with sucrose concentration from 2.5% to 20% have not been greatly encouraging. Concentration of 10% and greater have lead to leaf injury within 24 hours. The injury has taken the form of a marginal leaf water soak tissue appearance and premature leaf blackening. Lower concentration did marginally reduced the rate of browning. The significant difference from Brink's (1987) research method was that we subjected the flowers to a 2 days simulated packing.

4. Conclusion

The darkening of *Protea neriifolia* can be apparently triggered both by water stress and possibly a carbohydrate or other reserve shortage in the leaves. However, the role of the flower head in leaf blackening did not appear to be solely because of its role as a sink. Head removal and flower head girdling gave a similar delay in leaf blackening. It is possible that the head may contribute directly to initiation of leaf blackening.

These two triggers of leaf darkening might operate by their effect on

cellular membranes as suggested by Ferreira (1983). Attempts to measure this change would be difficult because of the high concentration of phenols present in the leaves that would be released during analysis.

The difference in rate of leaf blackening of flowers subjected and not subjected to a simulated packing period makes interpretation of some published data difficult. It would be worthwhile to know the underlying initiation factor in leaf blackening, but this should take into account commercial handling conditions.

The long term solution to this problem is to select clones that show less leaf blackening. Short term solutions are needed and these require an understanding of the preharvest factors that predispose some harvests to more rapid leaf blackening. The types of browning may be different with the tip, marginal and spot browning being one type and the wet steamy black leaf conditions found for some flowers on unpacking another.

5. References

Akamine, E.K., T. Goo and R. Suehisa. 1979. Relationship between leaf darkening and chemical composition of leaves of species of protea. Florist Rev. 163(4236):62-63,107-108.

Brink, J.A. 1987. The influence of the flower head on leaf browning of cut *Protea neriifolia*. Protea News No.5:11-12

Brink, J.A. and G.H. De Swardt. 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R. Br. Acta Hortic. 185:111-119.

De Swardt, G.H. 1980. Browning of foliage leaves in proteas. Fmg. S. Africa B.13:1-4.

De Swardt, G.H., J. Pretorus and L. Burger. 1987. The browning of foliage leaves in Protea - a review. Protea News No. 6:4-9.

Ferreira, D.I. 1983. Prevention of browning of leaves of *Protea neriifolia* R. Br. Acta Hortic. 138:273-276.

Ferreira, D.I. 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescence. Acta Hortic. 185:121-129.

Elsworth, J.F. and K.R. Martin, 1971. Flavonoids of the Proteaceae. Part 2. A chemical contribution to the studies on the evolutionary relationships in the South African Proteoideae Jour. Sth Afr. Bot 37:199-212.

Haasbrock, F.J., G.G. Rousseau and J.F. de Willeir. 1973. Effect of gamma rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamack and *Leucospermum cordifolium* Salisb. ex Knight. Agroplantae 5:53-62.

Ireland, J.P., J.T. Meynhardt and J.M. Strauss. 1967. When Proteas become sailors. Fmg. S. Africa 43(6):33-35.

Mulder, P.W.A. 1977. Primere meganismes betrokke by die bruinwording van loofblare in *Protea neriifolia*. MSC thesis, Rand Afrikaans University, Johannesburg.

Paull, R.E., T. Goo, R.A. Criley and P.E. Parvin. 1980. Leaf blackening in cut *Protea eximma*: Importance of water relations. Acta Hortic. 113:159-166.
Parvin, P.E., R.A. Criley and R.M. Bullock. 1973. Proteas: Developmental research for a new cut flower crop. HortSci 8:299-302.
Van Rheede van Oudtshoorn, M.C.B. 1963. Distribution of phenolic compounds in some South African Proteaceae. Planta Med. 11:300-406.

Table 1 Cultivar difference in number of days to 50% leaf blackening and to moderate flower bract curling for *P. neriifolia* flowers preconditioned overnight in Florever (20 g l^{-1}), packed dry for 3 days at 22°C and evaluated in Florever.

Clone	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Pink Splash	10 c	8 c
Rose Mink	24 b	35 a
Satin Mink	26 ab	16 b
Pink Mink	30 a	32 a

* Means in each column followed by the same letter do not differ statistically (P=0.05 Duncan/Waller Multiple range test).

Table 2 Seasonal changes in number of days to 50% leaf blackening and to moderate bract curling of *P. neriifolia* clone Pink Mink flowers preconditioned overnight in Florever (20 g l^{-1}), packed for 2 days at 22°C and evaluated in Florever.

Date of Harvest	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Sept. 22nd	17 c	12 c
Oct. 12th	34 a	22 a
Nov. 1st	28 b	18 b

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 3 Effect of stage of Pink Mink flower opening on days to 50% leaf blackening and moderate bract curling. Flowers were preconditioned in water overnight, packed for 3 days at 22°C and evaluated in deionized water.

Stage of Flower Opening	Days to 50% Leaf Blackening	Days Bract Curling
Closed Bud Stage	6 c	14 c
Bracts Just Unfolding	8 a	18 a
Cylindrical Shape	9 a	15 b
Conical Shape	8 a	14 c

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 4 Effect of flower removal and girdling the stem just below the flower on days to 50% leaf blackening of flowers held continuously in Florever (20 g l^{-1}).

Treatment	Days to 50% Leaf Blackening
Control	18 b
Flower Removal	24 a
Flower Girdle	24 a

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 5 Effect of antitranspirants on days to 50% leaf blackening of Pink Mink flowers preconditioned overnight in water after antitranspirant application, packed for 3 days at 22°C, and evaluated in water.

Treatment	Days to 50% Leaf Blackening
No Treatment	10 cd
Spray ABA 500ppm	11 bc
FMC-819 2% w/v	8 e
Folicote 5% v/v	12 ab
Polytrap 1% v/v	11 bc
Exhalt 20% v/v	12 a
Wilt Pruf 20% v/v	11 bc
Semperfresh 2% v/v	9 de

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 6 Effect of holding Pink Mink flowers continuously in light or darkness on rate of leaf blackening and to moderate flower bract curling. Flowers were held in deionized water.

Treatment	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Dark	10 b	11
Light (26 mE M^{-2} Sec^{-1})	16 a	11

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 7 Flowers were held for 24 hours in Floralife packed dry for various length of time then evaluated in water for days to 50% leaf blackening.

Period of Packing (days)	Days to Leaf Blackening
1	9 b
2	11 a
3	8 c
4	6 d

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

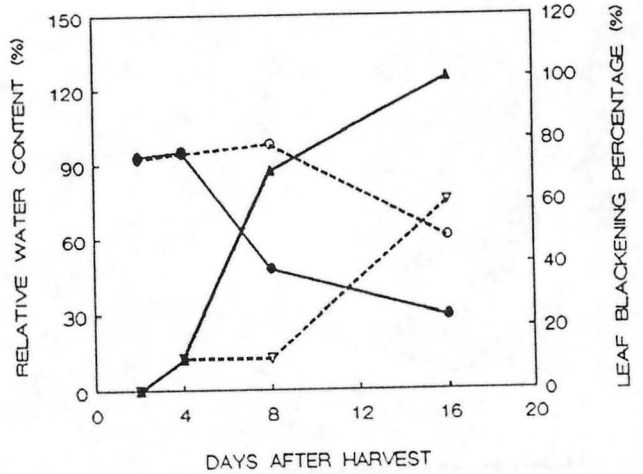

Figure 1 Postharvest changes in leaf relative water content and leaf blackening of *Protea neriifolia* cv Pink Mink. ●——● leaf relative water content of flower held in deionized water, ○- - - -○ leaf relative water content of flower held in Florever (20 g l^{-1}), ▲——▲ leaf blackening of flowers held in water, and ▽- - - -▽ leaf blackening of flowers held in Florever (20 g l^{-1}).

Levy, Y., Syvertsen, J.P., Nemec, S., 1983. Effect of drought stress and vesicular–arbuscular mycorrhiza on citrus transpiration and hydraulic conductivity of roots. New Phytologist 93, 61–66.

McGonigle, T.P., Miller, M.H., Evans, D.C., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytologist 115, 495–501.

Menge, J.A., 1983. Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. Can. J. Bot. 61, 1015–1024.

Modjo, H.S., Hendrix, J.W., 1986. The mycorrhizal fungus *Glomus macrocarpum* as a cause of tobacco stunt disease. Phytopathology 76, 688–691.

Mott, K.A., 1988. Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol. 86, 200–203.

Nemec, S., Menge, J.A., Platt, R.G., Johnson, E.L.V., 1981. Vesicular-arbuscular mycorrhizal fungi associated with citrus in Florida and California and notes on their distribution and ecology. Mycologia 73, 112–125.

Nemec, S., Guy, G., 1982. Carbohydrate status of mycorrhizal and nonmycorrhizal citrus rootstocks. J. Am. Soc. Horticul. Sci. 107, 177–180.

Nonami, H., Boyer, J.S., 1990. Wall extensibility and cell wall hydraulic conductivity decrease in enlarging stem tissues at low water potentials. Plant Physiol. 93, 1619–1619.

Peng, S., Eissenstat, D.M., Graham, J.H., Williams, K., Hodge, N.C., 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply. Plant Physiol. 101, 1063–1071.

Saab, I.N., Sharp, R.E., Pritchard, J., Voetberg, G.S., 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol. 93, 1329–1336.

Scott, T.A., Melvin, E.H., 1953. Determination of dextran with anthrone. Anal. Chem. 25, 1656–1661.

Stutz, J.C., Morton, J.B., 1996. Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. Can. J. Bot. 74, 1883–1889.

Syvertsen, J.P., Graham, J.H., 1990. Influence of vesicular arbuscular mycorrhizae and leaf age on net gas exchange of Citrus leaves. Plant Physiol. 94, 1424–1428.

Syvertsen, J.P., Lloyd, J.J., 1994. Citrus. In: Schaffer, B., Andersen, P.C. (Eds.), Handbook of Environmental Physiology of Fruit Crops, vol. II: Sub-Tropical and Tropical Crops. CRC Press, Boca Raton.

Watanabe, F.S., Olson, S.R., 1965. Test of ascorbic method for determining phosphorus in water and sodium bicarbonate extracts from soil. Soil Sci. Soc. Proc. 29, 677–678.

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The response of three *Leucadendron* cultivars (Proteaceae) to phosphorus levels

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Abstract

The objective of this study was to compare the growth of three *Leucadendrons* (Proteaceae) cultivars ('Safari Sunset', 'Orot' and 'Meir') fertigated with three levels of P. The plants were grown in tuff (pyroclastic material, characterized by high porosity) in 10 l pots and fertigated daily. The experiment included control treatment (plants irrigated with tap water) and three levels of P (0, 10 and 20 mg l^{-1}), while N and K in the P-treatments were kept at a constant rate of 50 mg l^{-1}.

Under P deficiency, the development of 'Orot' plants was significantly superior to that of 'Safari Sunset' and 'Meir' cultivars, but as P application increased to 20 mg l^{-1}, 'Safari Sunset' growth was quite similar to that of 'Orot'. The very low growth rate of 'Meir', probably eliminates this cultivar as a potential rootstock for commercial purpose. The low sensitivity of 'Orot' to nutrient application is a promising characteristic to avoid micro-nutrients deficiency and a significant advantage, especially under field conditions. No symptoms of P toxicity were observed even at the highest P level (20 mg l^{-1}), at any of the cultivars tested. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Leucadendron salignum*; *L. laureolum*; *L. coniferum*; *L. muirii*; *L.* 'Safari Sunset'; *L.* 'Orot'; *L.* 'Meir'; Nutrition; Proteaceae

1. Introduction

An effort has been made in Israel, in the last decade, to cultivate *Leucadendrons* 'Safari Sunset' and other Proteaceae (Proteas) as cut flowers

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(Ben-Jaacov, 1986; Ben-Jaacov et al., 1989). 'Safari Sunset' is a clonal selection of an artificial cross between *L. salignum* (red form) and *L. laureolum* and because of its great market value and relative ease of cultivation 'Safari Sunset' is the most important cultivar in the Protea industry. The production of 'Safari Sunset' is rapidly expanding, and Israeli export has increased from 1.3 million branches in 1992–1993 to 24 million in 1997–1998.

'Safari Sunset' was bred in New Zealand for the local acid soil and both of its parents are native to South African soils having low pH. Despite the suitable climate, growers of Proteas in Israel have encountered problems because of unfavorable soil characteristics, such as high pH, high free-lime content and in many cases too high level of phosphate. Two agro-technical methods are feasible for overcoming these soil limitations: (i) improving the rhizosphere conditions, and (ii) grafting sensitive cultivars on resistant rootstocks.

To improve the rhizosphere it is possible to supply preferable conditions in at least a restricted volume of the root zone by using a small volume of artificial substrate and/or by employing nutritional management that reduce the pH. A common practice in Israel for growing 'Safari Sunset' is to plant it in tuff, placed in holes dug in the native soil, or placed on the soil on a small pile. Tuff is a volcanic pyroclastic material, characterized by high porosity ($0.6\,l\,l^{-1}$) and high saturated hydraulic conductivity (Wallach et al., 1992). Using a high NH_4:NO_3 ratio and adequate nutritional management are common practices for achieving desirable pH and ion concentration in tuff medium and thus improving 'Safari Sunset' growth (Silber et al., 1998).

The idea of using rootstocks in Proteaceae (alternative (ii)) was suggested as early as 1966 by Rousseau (1966), but was commercially adopted only during the last decade (Ben-Jaacov et al., 1992). Some species, native to high pH soils in South Africa were studied as potential rootstocks in Israel in the late 1980s. As a result of these studies several clones were selected; the best clonal selection of *L. coniferum* was named 'Orot' and that of *L. muirii*, 'Meir'.

The possibility of improving plant production by using the two alternatives simultaneously, i.e., growing 'Safari Sunset' grafted on a resistant rootstock (alternative (ii)) in tuff medium and employing optimal nutritional management (alternative (i)) was suggested to be the most promising method. The growth of the new rootstocks must also be tested under intensive management of nutrient and water application. At present, insufficient information is available on the response of *Leucadendron* cultivars to nutrition management.

Growth reduction, leaf necrosis or chlorosis of the proteaceous plants are generally attributed to phosphorus toxicity (Buining and Cresswell, 1993; Goodwin, 1983; Nichols et al., 1979; Thomas, 1980). However, the improved growth of 'Safari Sunset' as a result of increasing water–P concentrations reported by Prasad and Dennis (1986) and Silber et al. (1998), contradict this general view and indicates that under intensive conditions when all nutrient

elements are supplied according to the plant needs, 'Safari Sunset' is probably not susceptible to realistic levels of soil-P concentrations. Using 'Orot' and 'Meir' as rootstocks for 'Safari Sunset' grown on unfavorable, high pH soils has been shown to be a good practical solution. (Ben-Jaacov et al., 1992). The response to fertilization management and the relative need and/or sensitivity of these cultivars and rootstocks to phosphate has not been evaluated. Thus, the response of a potential rootstock plant to different P concentrations is very important.

The objective of the present study was to compare the yield and plant growth of three cultivars of *Leucadendron* ('Safari Sunset', 'Orot' and 'Meir') to different P levels.

2. Materials and methods

The experiment was conducted in a screen-house at Bet Dagan, Israel (35°E, 31°N, 50 m alt.) irradiated by natural sunlight (10% shadow) and having a temperature range between 12°C and 35°C. 2 month-old rooted cutting of three cultivars, 'Safari Sunset' (*L. salignum* × *L. laureolum*), 'Orot' (*L. coniferum*) and 'Meir' (*L. muirii*), were grown in 10 l pots containing 0–8 mm-diameter tuff grains, between May and December 1992. The surface properties of the tuff used are similar to those reported by Silber et al. (1994).

The plants were fertigated daily with 1 l of the experimental nutrient solutions. The experiment included a control treatment (plants irrigated with tap water) and three levels of P: 0, 10 and 20 mg l^{-1} (P0, P1 and P2, respectively). The concentrations of N and K in these P treatments were both kept at a constant level of 50 mg l^{-1}, with NH_4:NO_3 = 1:1. Calcium and Mg were at the levels in the irrigation water used (50 and 20 mg l^{-1}, respectively). Micro-element concentrations (mg l^{-1}) applied were: Fe — 0.69, Mn — 0.34, Zn — 0.17, Cu — 0.025, Mo — 0.019 and B — 0.25, all EDTA-based, plus 2 mg l^{-1} Fe as EDDHA-Fe. The nutrient solutions were prepared from commercial fertilizers (NH_4NO_3, $(NH_4)_2SO_4$, KNO_3, and H_3PO_4) and typical Israeli tap water which contained (mg l^{-1}): 3 N-NO_3; 0.4 P; 6 K; 100 Na; 20 S; and 140 Cl. The electrical conductivity of the nutrient solutions was 1.2 ± 0.2 dS m^{-1} and their pH was 7.2 ± 0.4. Four single-pot replicates were arranged in a completely randomized design. At the end of the experiment the plants were collected and pooled for further analyses. The plants were washed with distilled water and divided into roots, stems and leaves. Fresh and dry (after drying at 60°C for 1 week) weights of the plant organs were determined. Dry plant material was ground to pass a 20-mesh sieve. Samples (100 mg) were wet ashed with H_2SO_4–H_2O_2 for analysis of Na, K, total N and P, or with $HClO_4$–HNO_3 for Ca and Mg.

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (SAS, 1985).

3. Result and discussion

3.1. Yield and plant growth

The three cultivars responded positively to nutrient addition when compared to the tap water, but the improvement rate differed among them. Addition of N, K and micro-nutrients (P0-plants) improved the dry weight of 'Safari Sunset' shoots 7.3-fold and that of the roots 5-fold when compared to the tap water (Table 1).

Phosphorus addition to 'Safari Sunset' nutrients at 10 mg l^{-1} (P1 treatment) significantly increased plant production by 30 ± 4% when compared to P0. Further P addition did not have significant growth improvement (Table 1). Shoot dry weight of 'Orot' plants irrigated with tap water was almost three times that of 'Safari Sunset' under the same conditions (Table 1). Nevertheless, the response of 'Orot' to nutrient addition was lower and less significant than that of 'Safari Sunset'. Thus, the dry weight (shoots and roots) production of 'Safari Sunset' fed with an adequate P level (P2) was quite similar to that of 'Orot' plants under the same conditions (Table 1). The dry weight production (shoots and roots) and the response to the fertilization treatments of 'Meir' plants were the lowest (Table 1).

Table 1
Treatment effects on the dry weights of shoots and roots of three cultivars of *Leucadendron*: 'Safari Sunset' (SS), 'Orot' (ORT) and 'Meir' (MR). Different letters within columns indicate significant difference for a single cultivar for the different fertilizer levels at the $P \leq 0.05$ level

Treatment	Shoot dry weight (g/plant)			Root dry weight (g/plant)		
	SS	ORT	MR	SS	ORT	MR
Water	11c	31b	8b	3c	4b	1.0b
P0	80b	122a	18a	15b	33a	1.8ab
P1	107a	135a	19a	19a	40a	1.8ab
P2	118a	132a	21a	21a	28a	2.5a
Analysis of variance						
Mean	79	105	17	15	26	1.8
F-trt[a]	52***	10**	5.4*	339***	13**	NS[b]
LSD-trt[c]	22.6	50.1	8.1	2.1	13.5	1.5
F-pl[d]	57***			96***		
F-pl.*trt[e]	4.8**			9.9***		
LSD-pl.[f]	17			3.6		

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
[a] F-trt — F test between treatments on each cultivar.
[b] NS — not significant.
[c] LSD-trt — LSD test between treatments on each cultivar.
[d] F-pl — F test between the three cultivars.
[e] F-pl.*trt — F test of the mutual interaction of cultivar and treatment.
[f] LSD-pl. — LSD test between the three cultivars.

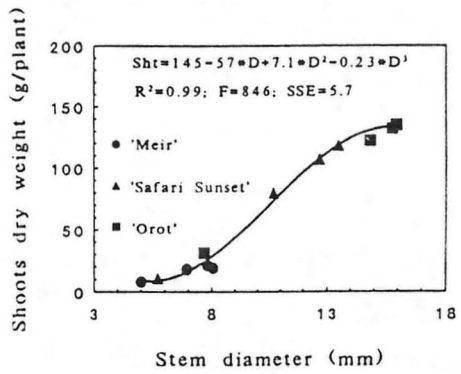

Fig. 1. Shoot dry weight (Sht (g/plant)) as a function of stem diameter of all three cultivars (*D* (mm)) at the end of the experiment. The line was calculated by means of the equation presented and the experimental points.

3.2. Stem diameter

Significant linear regressions were obtained between stem diameter and shoot dry weight of each cultivar at the end of the experiment ($R^2 = 0.92$, 0.99 and 0.99 for 'Meir', 'Safari Sunset' and 'Orot', respectively, data not presented.) A polynomial equation best described the relationships between stem diameter and shoot dry weight of the three cultivars together (Fig. 1). Stem diameter expanded during the experiment according to the nutritional management (Fig. 2).

The correlation between stem diameter (*D* (mm)) and the time elapsed from the beginning of the experiment (*t* (days)) could be described by a linear equation. Tap-water-fed plants had the smallest stem diameter during the experiment, and it expanded slowly in comparison with the other treatments (Fig. 2). Similar to dry weight production, the rate of stem diameter expansion also increased as a result of the addition of N and K (P0) and increased further in response to the addition of P. The responses of 'Orot' and Meir' cultivars to the addition of P were less dramatic than that of 'Safari Sunset' (Fig. 2).

3.3. Element concentrations in plants

Plant responses to nutritional management differed significantly by cultivar. Similarly to growth parameters, the response of 'Safari Sunset' to fertilization management exceeded those of 'Orot' and 'Meir'. Nitrogen addition to 'Safari Sunset' plants increased N concentration in leaves by 280% (tap-water-fed

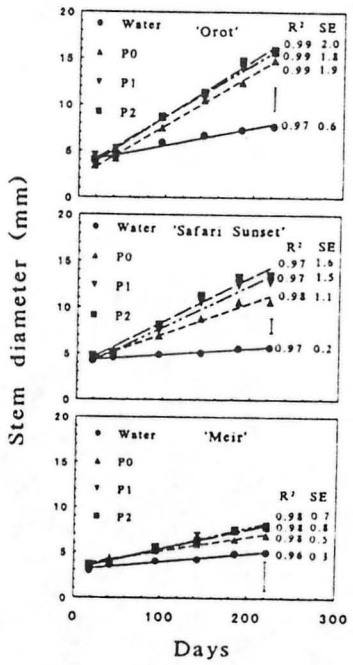

Fig. 2. Stem diameter (D (mm)) of the three *Leucadendron* cultivars as a function of time (t (days)). The lines were calculated according to the equation: $D = A + b \times t$; the parameters were significant at $P \leq 0.05$. Vertical lines at the right indicate $LSD_{0.05}$ of stem diameter measured at the end of the experiment for each cultivar. $LSD_{0.05}$ for the differences between cultivars was 1.12.

compared with P0-fed plants), while in 'Orot' and 'Meir' the increases were only by 50% and 76%, respectively (Table 2). The increases of N concentrations in the roots due to N addition (tap-water-fed compared with P0-fed plants) was 360%, 200% and 170%, for 'Safari Sunset', 'Orot' and 'Meir' cultivars, respectively.

The low N content and the weak response to fertilization management of the 'Meir' cultivar (Table 2) were in accord with its low dry mass production (Table 1). However, in the case of 'Orot', which had the highest dry mass production, N concentrations in leaves were almost the same as those in 'Meir'. Addition of P (P1) enhanced N concentrations only in 'Orot' leaves, but further P addition (P2) did not have significant effect on N concentration (Table 2).

Red tuff contains 4.6% Ca–P minerals (Silber et al., 1994). Phosphorus uptake by zero-P-fed-plants (P0), especially by the 'Safari Sunset' cultivar, may result from the P dissolution from the indigenous tuff sources because of organic acid exudation (Silber et al., 1998). The responses of the P content in leaves to P nutrition had a similar trend for all three cultivars, e.g., P concentrations in the

Table 2
Treatment effects on N, P, K, Ca and Mg concentrations (g kg^{-1} DW) in leaves and roots of three cultivars of *Leucadendron*: 'Safari Sunset' (SS), 'Orot' (ORT) and 'Meir' (MR)[a]

Treatment	N			P			K			Ca			Mg		
	SS	Ort	MR	SS	Ort	MR	SS	Ort	MR	SS	Ort	MR	SS	Ort	MR
Leaves															
Water	10b	10c	8.5b	1.8c	0.7c	0.4b	10.8 a	7.1a	7.0c	12.0a	9.9ab	3.7	2.0	5.4a	2.4a
P0	28a	15b	15a	1.9c	1.1c	0.6b	8.0b	4.9b	8.9b c	8.9b	8.8bc	4.0	1.8	3.7bc	2.0b
P1	26a	19a	18a	4.9b	4.4b	1.3a	9.0b	5.2b	9.9b	9.2b	8.1c	3.9	2.1	3.0c	2.2ab
P2	26a	18ab	15a	8.2a	9.1a	1.5a	7.3b	6.6a	13.3a	8.8b	10.4a	3.7	1.7	3.7bc	2.2ab
Analyses of variance															
Mean	22	16	14	4.2	3.9	1.0	8.8	6.0	9.8	9.7	9.3	3.8	1.9	3.9	2.2
F-trt	44***	20***	17***	479***	364***	63***	6.9*	9.3**	11**	32***	5.6*	NS[b]	NS	24***	NS
LSD-trt	3.9	2.8	3.1	0.5	0.7	0.2	2.2	1.1	2.5	0.86	1.4	0.7	0.54	0.65	0.39
F-pl	66***			847***			40***			415***			187***		
F-Pl*trt	7.5***			146***			12***			10***			11***		
LSD-pl	1.6			0.3			0.9			0.47			0.23		
Roots															
Water	5c	7b	10b	1.3c	1.1c	0.8b	7.5a	5.5	5.4a	9.8a	7.6	3.0	2.3b	5.2	4.7a
P0	18b	14a	17a	1.5c	1.1c	1.2b	6.6ab	6.5	5.8a	6.4b	7.7	2.8	3.5a	3.4	1.7b
P1	20ab	15a	16a	3.1b	2.3b	3.1a	5.8b	6.1	5.3a	3.4c	9.6	3.3	2.0b	4.0	2.1b
P2	23a	16a	15a	4.6a	3.6a	3.2a	4.5c	5.3	4.5b	4.1c	8.4	3.7	3.4a	4.1	3.9a
Analyses of variance															
Mean	16	13	14	2.6	2.0	2.0	6.1	5.8	5.2	5.9	8.3	3.2	2.8	4.2	3.1
F-trt	66***	11***	13***	95***	167***	72***	14***	NS	6.3*	73***	NS	NS	5.7*	NS	27***
LSD-Tr	3.1	3.7	2.8	0.5	0.3	0.5	1.1	1.4	0.7	1.06	4.2	1.4	1.0	2.5	0.9
F-pl	24***			9.6***			66***			415***			187***		
F-pl*trt	NS			8.0***			4.6***			10***			11***		
LSD-pl	1.6			0.4			1.1			0.47			0.23		

* Significant at $P \leq 0.05$.
** Significant at $P < 0.01$.

leaves and the roots increased as a result of increasing P levels in the irrigation water (Table 2). Generally, the P content in the roots of 'Meir' plants were at the same level as those of the other two cultivars (except for P2, Table 2), which suggests that the low P content in leaves of 'Meir' did not result from low P uptake by the root system. During the experiment there was no indication of toxicity symptoms that could be attributed to an excess of P.

The K concentrations in the leaves and roots of all three cultivars were very low (Table 2) compared with those in other ornamental plants (Jones et al., 1991), which was consistent with the results of Parks et al. (1996) for several proteaceous plants and of Silber et al. (1998) for 'Safari Sunset' plants. The low K requirement of proteaceous plants may be attributed to an adaptation mechanism to the low-K soils on which they originated, as suggested previously by Parks et al. (1996). Despite zero-K fertilization, the K contents in leaves of tap-water-irrigated 'Safari Sunset' and 'Orot' plants were higher than those in leaves of plants irrigated with nutrient solution (Table 2). This may be a result of dilution or of competition with NH_4^+ on the root uptake sites. Sodium concentrations in leaves were very high and exceeded those of K (on a molar basis) in all treatments (8.9 ± 2, 4.8 ± 0.5, and 4.8 ± 0.5 mg kg^{-1}, for 'Safari Sunset, 'Orot' and 'Meir', respectively). High Na content in the leaves might be a result of K replacement, a mechanism which is well known in halophytes, although the amount of K replaced is very small (Marschner, 1995). Similar to K contents and probably by the same reasons, the Ca concentrations (leaves and roots) in 'Safari Sunset' plants decreased in plants fed by nutrient application (Table 2). In 'Orot' and 'Meir' plants the fertilization treatments did not affect the Ca and the Mg concentrations in the leaves and in the roots. Mg concentrations decreased following fertilization only in 'Orot' leaves and in 'Meir' roots.

4. Conclusions

The growth of 'Meir' plants was very low, under all the regimes applied, which probably eliminates 'Meir' as a potential rootstock for the purpose of cut flower production. 'Meir' may have a potential as a dwarfing rootstock for *Leucadendrons*. The development of 'Orot' plants under P deficiency was significantly superior to that of the other two cultivars. The fact that 'Orot' was not affected by P levels, may be an advantage while using it as rootstock under variable soil conditions. However, the improvement of dry mass production of 'Safari Sunset' plants as a result of the nutrient solution application was the highest and, therefore, under appropriates P concentration application (20 mg $P\,l^{-1}$), the yields of 'Safari Sunset' and 'Orot' plants were almost the same. In order to achieve an optimal management regime for 'Safari Sunset' and 'Orot', it is important to perform additional examinations including the responses to N

application rates and to water management (water uptake and frequency of irrigation). Nevertheless, the insensitivity of 'Orot' plant to P applications is a promising characteristic which may be used to avoid micro-nutrients deficiency and could offer significant advantages, especially under field conditions. Improving *Leucadendron* production can be achieved by combining proper soil conditions (addition of tuff), with the use of rootstocks and optimal nutritional application.

References

Ben-Jaacov, J., 1986. Protea production in Israel. Acta Hortic. 195, 101–110.
Ben-Jaacov, J., Ackerman, A., Gilad, S., Schori, Y., 1989. New approaches to the development of Proteaceous plants as floricultural commodities. Acta Hortic. 253, 193–199.
Ben-Jaacov, J., Ackerman, A., Gilad, S., Carmeli, R., Barzilay, A., Schori, Y., 1992. Grafting techniques and the use of rootstocks in *Leucadendron*, and other Proteaceous plants. Acta Hortic. 316, 69–71.
Buining, F., Cresswell, G., 1993. Working party on nutrition Proteaceae. J. Int. Protea Ass. 26, 21–27.
Goodwin, P.B., 1983. Nitrogen, phosphorus, potassium and iron nutrition of Australian native plants. In: Proc. Nat. Tech. Wkshp on Prod. and Markg. of Australian Wild Flowers for Export. Univ. Ext., Univ. West., Nedlands, pp. 85–97.
Jones Jr., J.B., Wolf, B., Mills, H.A., 1991. Plant Analysis Handbook. Micro-Macro Publishing, GA.
Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, San Diego, CA.
Nichols, D.G., Jones, D.L., Beardsell, D.V., 1979. The effect of phosphorus on the growth of *Grevillea* 'Poorinda Firebird' in soil-less potting mixtures. Sci. Hort. 11, 197–206.
Parks, S.E., Cresswell, G.C., Haigh, A., Buining, F., Barlow, E.W.R., 1996. Nutritional requirements of some Proteaceous plants. In: IV National Workshop for Australian Native Flowers.
Prasad, L., Dennis, D.J., 1986. Phosphorus nutrition of *Leucadendron* 'Safari Sunset'. Acta Hort. 185, 155–162.
Rousseau, G.G., 1966. Proteas can be grafted. Farming in S. Africa 42(6), 53–55.
SAS, 1985. SAS User's Guide. SAS Inst., Cary, NC, USA.
Silber, A., Bar-Yosef, B., Singer, A., Chen, Y., 1994. Mineralogical and chemical composition of three tuffs from Northern Israel. Geoderma 63, 123–144.
Silber, A., Ganmore-Neumann, R., Ben-Jaacov, J., 1998. Nutrient effects on growth and rhizosphere pH of *Leucadendron* 'Safari Sunset' development. Plant Soil 199, 205–211.
Thomas, M.B., 1980. Phosphorus response of Proteaceae and other nursery plants in container. Ann. J. Royal New Zealand Inst. Hort.
Wallach, R., da Silva, F.F., Chen, Y., 1992. Hydraulic characteristics of tuff (scoria) used as a container medium. J. Am. Soc. Hort. Sci. 117, 415–421.

thin, up to 2,5 mm diam., yellowish to light-brown flesh, non-fibrous. *Leaves* incurved, erect, firm, up to 27 mm long, 7 mm broad, 1,5 mm thick, subovate lanceolate acuminate, shortly aristate with bristle up to 1 mm long, dark green above, whitish below, surface minutely granulate, marked with longitudinal lines and a few transverse veins; *face* slightly convex at base, 3–4 longitudinal lines; *back* convex, 4–6 faint longitudinal lines, strongly keeled from base, from near middle when turgid, keel with up to 8 reflexed spines usually in 1 but occasionally in 2 parallel rows; *margins* acute, spined in upper half, occasionally from 1/3; *spines* translucent white, up to 1,2 mm long, 1–2 mm apart. *Peduncle* simple, 0,75–1 mm diam., 30–35 cm long including raceme, pinkish-brown; *base* 2-angled; *raceme* 8–10 cm long, 5–12 flowers, 1–2 open; *pedicels* 0,5 mm diam., 3–5 mm long; *sterile bracts* 7–11, 4–6 mm long; *fertile bracts* 3–5 mm long. *Perianth* white, thin brownish nerves, 14–15 mm long, upper outer segments suberect, very lightly plicate at tips, inner lower segments with upper margins strongly incurved proximally, tube very pale green; *buds* narrow elongate, arcuate, younger buds rounded at tips. *Flowering* Dec.

The affinities of *H. pulchella* are uncertain. Vegetatively, the nearest relative may be *H. monticola* Fourcade from the Molen River–Herold area which, however, has the long narrow leaves of the section *Loratae* S.D. The rather narrow elongate buds and tepals are also indicative of affinity with *H. monticola*. The compact growth form is characteristic of *H. marumiana* Uitew. and is also found in *H. herbacea* (Mill.) Stearn and some forms of *H. schuldtiana* V. Poelln. *H. pulchella* is quite at variance with these species as far as nuances of leaf colour, marking and texture are concerned. Of particular note is that this species is geographically well separated from possible allies although it does grow in close proximity to *H. setata* Haw. *H. pulchella* is found in the area extending from Avondrust to Nougaspoort, southeast of Touws River. It was first recorded from Avondrust by the late Mr R. C. Littlewood and recollected by Mr F. J. Stayner and the writer.

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ROOT AND CROWN ROT OF SILVER TREES*

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Abstract

Greenhouse and field inoculations showed *Phytophthora cinnamomi* Rands to be the primary cause of root and crown rot, and subsequent dieback of the silver tree, *Leucadendron argenteum*. Other related hosts were also recorded, though certain *Protea* spp. appear to be tolerant to the disease.

Uittreksel

WORTEL EN KRAAGVERROTTING BY SILWERBOME

Inokulasies in die glashuis en in natuurlike omgewings het getoon dat *Phytophthora cinnamomi* Rands primêr verantwoordelik is vir die wortel- en kraagverrotting en die gevolglike afsterwing van silwerbome, *Leucadendron argenteum*. Verwante gashere is ook aangetoon, alhoewel sekere *Protea* spp. verdraagsaamheid teen die siekte openbaar het.

Introduction

Silver trees (*Leucadendron argenteum*) are subject to a sudden dieback which is of common occurrence throughout the Western Cape. The first indication of disease is the wilting of the entire tree, but removal of the external bark of recently killed trees exposes a dark patch of rotted bark extending from the crown into the root (Fig. 1). This browning may also occur on some apparently healthy trees but is masked by the external bark.

Olivier (1951) and Wager (1970) ascribed dieback of silver trees to infection by the fungus *Botryosphaeria ribis* (Tode ex Fries) Gross & Dugg. However, Olivier's (1951) work was inconclusive in that inoculations of fully grown silver trees with *B. ribis* consistently failed to induce the disease.

The present investigation was carried out to establish the primary cause of dieback of silver trees.

Material and methods

Isolation

Naturally infected plant material from Stellenbosch, Banhoek, Paarl, Somerset West and Kirstenbosch was plated out on corn meal agar after surface

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* Results to be submitted in partial fulfilment for a Ph.D. degree at the University of Stellenbosch.

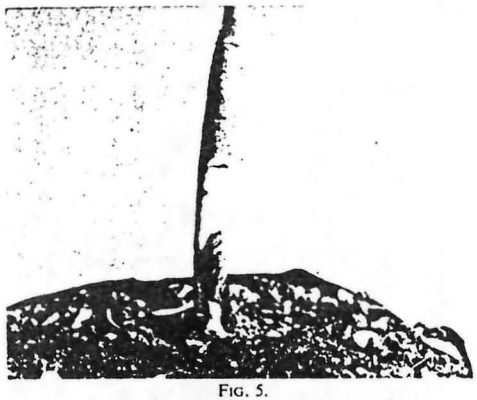

FIG. 5.
Lesion development on a young potted silver tree three months after inoculation with *Botryosphaeria ribis*. External bark removed.

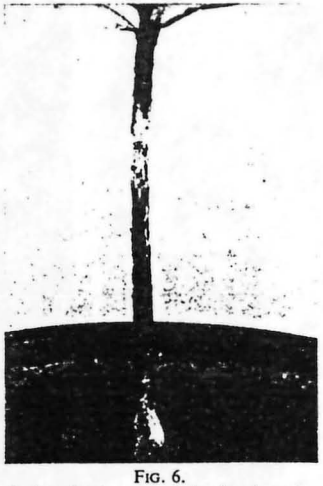

FIG. 6.
Lesion development following inoculation of a young silver tree with *Phytophthora cinnamomi*. External bark removed.

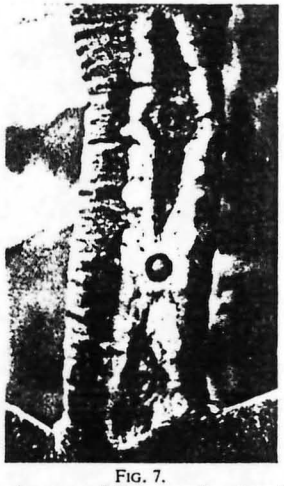

FIG. 7.
Lesions on a full grown silver tree 10 days after inoculation with *Phytophthora cinnamomi*. Control inoculation in the centre. External bark removed.

Inoculations

Greenhouse inoculations

All plants inoculated with *P. cinnamomi* developed brown lesions similar to those observed in naturally-infected trees. Plants at low and fluctuating temperatures developed the brown discolouration only at the inoculation sites but none died within a period of three months after inoculation. In contrast, all plants held at high temperatures died within 10–12 days (Fig. 4).

Inoculations with *B. ribis* were also positive, though the browning was limited (Fig. 5) and contrasted markedly with the progressive browning (Fig. 6) caused by *P. cinnamomi*. None of the plants inoculated with *B. ribis* died within the observation period of three months.

Inoculations of plants in their natural habitat

All trees growing in their natural habitat and inoculated with *P. cinnamomi* developed typical browning around the point of inoculation (Fig. 7). This was usually followed by rapid wilting of the tree. The younger trees died within ten days of inoculation, while older trees survived for longer periods. After 12–15 weeks only the oldest tree, which died two months later, remained alive.

Inoculation of silver trees with *B. ribis* was also successful, although limited lesion development began only 12–15 weeks after inoculation. No trees died within this period.

Both the *Leucospermum cordifolium* and *L. glabrum* plants died within ten weeks of inoculation with *P. cinnamomi*, the symptoms being typical of those on naturally-infected plants.

Re-isolation

Both *P. cinnamomi* and *B. ribis* were consistently re-isolated from lesions developing after inoculation with the respective organism.

Occurrence of P. cinnamomi *on Proteaceae*

Phytophthora cinnamomi was isolated from the crown and root tissues of recently-killed plants of the following species growing in their natural habitat or under cultivation.

Leucadendron argenteum
L. tinctum
L. salicifolium
Leucospermum cordifolium
L. reflexum
L. lineare
L. catherinae

Preliminary investigations indicate that certain *Proteas* pp., (eg *P. cynaroides*), are tolerant to this disease.

Discussion

Phytophthora cinnamomi has an extremely wide host range which includes many Proteaceae (Zentmeyer & Thorn, 1967; Robertson, 1969; Weste & Taylor, 1971; Davison, 1972; Podger, 1972; Newhook & Podger, 1972). The present investigation has shown that it also causes a sudden dieback of silver trees, and that the dieback occurs at high temperatures. The effect of high temperatures in enhancing disease development was also noted in Australia where *P. cinnamomi* is particularly destructive to local Proteaceae during the spring and summer months (Newhook & Podger, 1972). It is therefore postulated that *P. cinnamomi* is the pathogen responsible for the common dieback of silver trees and certain other Proteaceae in South Africa.

Botryosphaeria ribis, although a major pathogen, is not regarded as a primary cause of dieback of silver trees.

Although the full host range of *P. cinnamomi* among South African Proteaceae has not been determined, it appears that certain *Protea* spp., notably *P. cynaroides*, are tolerant to the disease. The nature of this resistance is at present under investigation.

References

Davison, Elaine M., 1972. *Phytophthora cinnamomi* on ornamentals in South Australia. *Pl. Dis. Reptr* 56: 290.

Newhook, F. J. and Podger, F. D., 1972. The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. *Ann. Rev. Phytopath.* 10: 299–326.

Olivier, Dorothea, 1952. Progress in the study of the silver-tree disease. *J. bot. Soc. S.A.* 37: 18–19.

Podger, F. D., 1972. *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. *Phytopathology* 62: 972–981.

Robertson, G. I., 1969. Susceptibility of exotic and indigenous trees and shrubs to *Phytopthora cinnamomi* Rands. *N.Z. Jl agric. Res.* 13: 297–307.

Sewell, G. H. F. and Wilson, J. F., 1959. Resistance trials of some apple rootstock varieties to *Phytophthora cactorum* (L & C) Schroet. *J. hort. Sci.* 34: 51–58.

Van der Merwe, J. J. H. and Matthee, F. N., 1972. *Phytophthora* root rot of grapevines in the Western Province. *Decid Fruit Grow* 22: 268–269.

Wager, V. A., 1970. *Flower garden diseases and pests.* Cape Town: Purnell.

Weste, Gretna M. and Taylor, P., 1971. The invasion of native forest by *Phytophthora cinnamomi*. 1. Brisbane Ranges. *Aust. J. Bot.* 19: 281–294.

Zentmyer, G. A. and Thorn, W. A., 1967. Hosts of *Phytophthora cinnamomi*. *Calif. Avocado Soc. Yearbook* 51: 177–186.

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AN EVALUATION OF TECHNIQUES USED FOR EXTRACTING ENDOGENOUS CYTOKININS FROM PLANT MATERIAL

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Abstract

Using paper and column chromatography it was found that considerable amounts of cytokinins can be lost from aqueous plant extracts during purification by solvent partitioning. Most, if not all, of the free base cytokinins present in seed extracts of *Rumex obtusifolius* partitioned into petroleum ether at pH 9,0 and ethyl acetate at pH 2,5. Whether the presence of these compounds will be detected by paper chromatography depends very much on the solvent system used.

Uittreksel

'N EVALUASIE VAN TEGNIEKE OM SITOKINIENE UIT PLANTMATERIAAL TE EKSTRAHEER.

Met behulp van papier- en kolomchromatografie is gevind dat wanneer plantekstrakte met behulp van organiese oplosmiddels gesuiwer word, relatief groot hoeveelhede van die sitokiniene verlore gaan. Die meeste van die vrye basis sitokiniene in saadekstrakte van *Rumex obtusifolius* beweeg byvoorbeeld in petroleumeter, pH 9,0, en etielasetaat, pH 2,5, in. Of die teenwoordigheid van sodanige verbindings met behulp van papierchromatografie aangetoon sal word, hang grotendeels af van die chromatografiese oplosmiddels wat gebruik word.

Introduction

The endogenous cytokinins extracted from plant material with aqueous methanol or ethanol are usually purified to some degree prior to being bioassayed. This is frequently done by partitioning the extracted substances with one or more organic solvents at different pH values.

Pigments and fatty substances have been removed from aqueous plant extracts, at pH 9,0, by partitioning with petroleum ether (Gazit and Blumenfeld, 1970: Prakash and Maheshwari, 1970). These authors could detect no cytokinin activity in the petroleum ether fraction. However, Van Staden and Wareing (1972) have reported cytokinin activity in such extracts. As a number of synthetic cytokinins did not partition into petroleum ether at pH 9,0 it was, at the time, suggested that the activity was perhaps due to substances that are less polar than the substituted adenines which constitute the known cytokinins. This however, does not mean that the natural cytokinins would behave similarly.

In order to remove acidic inhibitors, gibberellins and auxins from plant extracts the pH values of these extracts are adjusted to 2,5 before partitioning against ethyl acetate. That no cytokinin activity could be detected in the ethyl acetate fraction (Gazit and Blumenfeld, 1970; Mayak and Halevy, 1970; Van Staden and Wareing, 1972) is perhaps not surprising as they will contain phenolic compounds as well as inhibitors that would have an adverse effect in most

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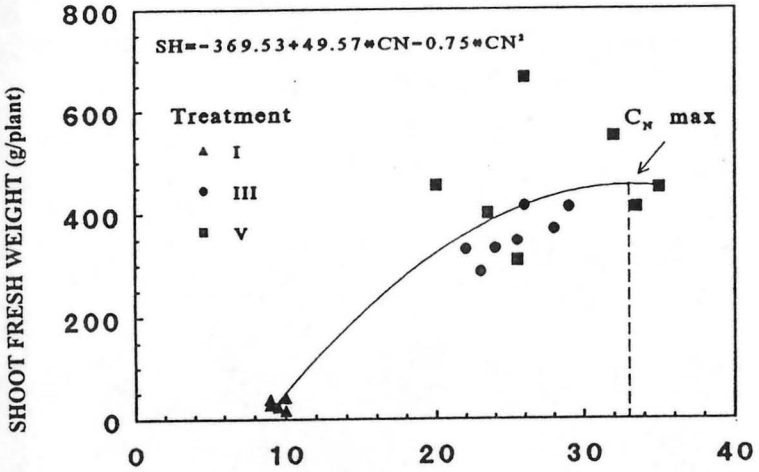

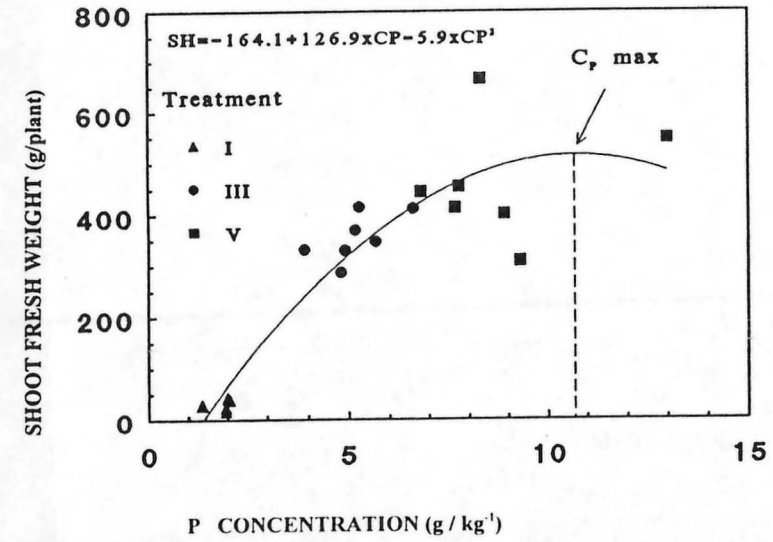

Fig. 7. Shoot fresh weight (SH; g plant^{-1}) as a function of N and P concentrations (CN and CP, respectively; g kg^{-1} dry matter) in mature leaves. The lines were calculated according to second-order equations (Table 4) and the experimental points in Table 3.

EFFECT OF pH ON THE DEVELOPMENT OF *LEUCADENDRON* 'SAFARI SUNSET'

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Abstract

Proteaceous plants grow naturally on acidic washed soils in Australia and South-Africa therefore their cut flower production is difficult in Israel., where soils are mostly basic and calcareous. An experiment was conducted in an aerohydroponic system to establish the effect of pH on the development and growth of *Leucadendron* 'Safari Sunset'. Total fresh weight, root fresh weight percentage and N and P where concentrations decreased significantly in plants grown in high pH (7.0) solutions compared to plants grown in low pH solutions (5.5). The high pH inhibited root growth and subsequent shoot growth. A marked effect of the pH on the proliferation of root hairs was demonstrated by using a scanning electron microscope. In roots of plants grown at high pH, root hair development was arrested, thus decreasing the potential surface area, which may decrease in plant nutrient uptake. L. 'Safari Sunset' required low pH in its rhizosphere for adequate growth, as root hairs developed only at pH lower than 6.

1.Introduction

Proteaceae family originated from Australia and South Africa, where most of the species grow on acid washed soils, poor in nutrients. Most of the species in the family have a double root system: proteoid and normal root systems. Much attention has been directed to the role of proteoid roots in the growth and development of plants (Lamont, 1972). Proteoid, short life, roots, are regarded as an alternative system for enhancing nutrient uptake in poor soils (Lamont, *et al.*, 1984; Lamont, 1986). They increase root surface thus increasing the absorbing and exuding area of the root system. Proteoid roots formation is suppressed by high nutrient availability. Roots hairs, although differ in their developmental origin, can be compared to proteoid roots in their life length and contribution to the root surface area (Hofer, 1995). Root hair size and development is influenced by environmental factors such as pH (Ewens and Leigh, 1984) and ion concentration (Marschner and Romhed, 1995). PH has a direct effect on growth and root elongation, as was documented by Tan, *et al.* (1993) and Yan, *et al.* (1992)

In Israel, an effort was made in the last decade to cultivate Proteaceae species for cut flowers (Ben-Jaacov, 1986; Ben-Jaacov, *et al*, 1989). Although the climate is suitable, the soils are mostly calcareous, with high pH. The commercial cultivation raised the question if low pH in the rhizosphere is necessary for plant development.

The purpose of the experiment was to establish the effect of pH on the development and growth of *Leucadendron* 'Safari Sunset'

2. Materials and methods

The experiment was conducted in a screen house in Bet Dagan, Israel (35°E, 31°N, 50 m altitude) irradiated by natural sunlight (1400-1600 µmol m^{-1} PPF) at a temperature range between 12-35°C.

Two months old *Leucadendron* 'Safari Sunset' plants were transplanted on 6 June 1993 into an aerohydroponic system (Feigin, *et al.*, 1984), consisting of two separate 50 cm x 29 cm x 20 cm deep polystyrene boxed mounted on a 140 l container (1 plot). Roots were

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exposed continuously to the nutrient solution which was circulated by means of a plastic tube system with small holes through which the solution was ejected. The solution leached to the bottom of the container and recirculated continuously. The experiment was arranged in randomized blocks with 5 replicates each block had 12 plants.

The experiment consisted of six treatments in a non factorial design (Table 1): two levels of P (7 and 20 mg/l), two NO_3/NH_4 ratios (40/60 and 60/40) and two pH levels (5.5 and 7.0).

Micro nutrients and Fe were: Mn, 0.234; Cu 0.025; Zn,0.18; Mo, 0.02, B, 0.25 and Fe, 0.67 mg/l as EDTA chelate and 2.0 mg/l Fe as Sequestrin. The nutrient solutions were prepared with commercial fertilizers: KNO_3, $(NH_4)SO_4$, NH_4NO_3, KCl, KH_2PO_4, Koratin and Sequestrin added to tap water consisting of approximately Na, 100; P, 0.4, NO_3 10; Ca, 50; Mg, 20 and Cl, 140 mg/l. The pH was monitored daily. To maintain low and high pH, H_2SO_4 and NaOH was added respectively. The electric conductivity was 2 ddS/m and did not change significantly by adding H_2SO_4 or NaOH.

The solutions were renewed weekly and daily loss of water was returned.

At the end of the experiment, 25 August, 1993 plants were removed and divided into root and shoot, fresh and dry weight were determined in the plant organs.

Scanning electron microscopy pictures were taken from roots of plants grown in nutrient solution, at the two pH treatments.

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (SAS, 1985) Different letters in table indicate significant difference at the $p<0.05$ level.

3. Results and discussion

3.1. Yield

Plant total fresh weight, root fresh weight and percentage of root weight of total weight, of L. 'Safari Sunset' as affected by pH, P concentration and NH_4/NO_3 ratio are presented in Table 2.

The maximum fresh matter production (74.23 g/pl) was obtained in treatment I (pH-5.5, P-7 mg/l and NH_4/NO_3 ratio of 60/40), while the minimum fresh matter production (26.98 g/pl) was obtained in treatment V (pH-7.5, P-20 mg/l and NH_4/NO_3 ratio of 60/40). Thus, growth response was about threefold greater comparing both treatments. Total fresh weight, and root fresh weight of plants grown in high pH (7) solutions were significantly low compared to plants grown in low pH solutions (5.5). The high pH inhibited primarily root growth and subsequently shoot growth.

Decreasing the NH_4/NO_3 ratio from 60/40 (treatments I and IV) to 40/60 (treatments III and VI) decreased the yield from 74.23 g/pl to 59.38 g/pl in the presence of low pH. The preference for high NH_4/NO_3 ratio in the solution was also reported by Heinson and Paramenter (1985) for *Leucadendron salignum* Berg grown in water culture. Increasing P concentration in the nutrient solution from 7 mg/l to 20 mg/l resulted in a decrease of total and root fresh weight. The effect was stronger in the plants grown in low pH.

Percentage of root weight from total plant weight was lower in the treatments with high pH (18.5-23.0) compared to treatments with low ph. treatments (22.4-29.6). The inhibited root probably affected plant development.

3.2. Root morphology

3.2.1. Root system morphology

The effect of pH on root development is presented in Plate 1. The root development of plants grown at high pH is restricted because of poor branching and dead roots (black roots). No proteoid roots, which develop for improving nutrient uptake were observed since the ion composition and concentration were not limiting factors. In the presence of sufficient ion concentration no proteoid rood developed as was reported for *L.* 'Safari sunset' (Silber, *et al*, 1996) and for other species (Lamont, 1982; Racette, *et al*. 1990).

3.2.2. Development of root hairs

The effect of pH on the development of root epidermal cells and root hairs using scanning electron microscope is presente din Plate 2. Root hair development was inhibite in plants grown at high pH. This decreased the potential surface area, which may lead t the decrease in plant mineral uptake (direct effect of pH) or exuding of organic acids t the rhizosphere (indirect effect of pH).

3.2.3. Cell elongation

High pH affected root length probably by inhibiting cell elongation. Cells wer measured for length and width at 1 mm distance from the root tip. The low pH root cell were longer (30-40 μm) compared to high pH root cells (20-32 μm) but there width wa similar (6-8 μm).

The reduction of 'safari sunset' root growth at pH of 7.0 could be attributed t inhibition of cell elongation without affecting cell division, as was also reported for lupin roots (White, 1990; Tang, *et al*., 1993). The mechanism by which high pH impairs cel elongation and restriction of root hair formation is unknown. Cell wall acidification causing loosening of cellulose microfibrils in the walls is suggested to be the cause o optimal cell growth (Taiz, 1984).

4. Conclusions

Poor growth of L. 'Safari sunset' on alkaline soils has been reported but there are n previous studies on the specific effect of high pH. The present study shows that high pl inhibited root growth and decreased shoot plant weight. *L.* 'Safari sunset' required low pl in its rhizosphere for adequate growth, as root hairs developed only at pH lower than 6.

References

Ben-Jaacov, J., 1986. Protea production in Israel. Acta Hort.. 185, 101-110.

Ben-Jaacov, J., Ackerman, A., Gilad, S. and Shchori, Y. 1989. New approaches to th development of Proteaceous plants as floricultural commodities. Acta Hort. 253, 193-199.

Ewens, M. and Leigh, R.A., 1985. The effect of nutrient solution composition on th length of root hairs of wheat (*Triticum sativum* L) J. Exp. Bot. 36, 713-724.

Feigin, A., Zamir, N., Arbel, A. and Kilman. A. 1984. A closed hydroponic system FO experiments with plants growing in circulating nutrient solution. Proc. 9th Int. Congr. Soilles Culture, 215-223.

Heinsohn, R. D., and Pamenter, N.W., 1986. A preliminary study of interactions between nitrogen, potassium and phosphorus in the mineral nutrition of seedlings of *Leucadendron salignum* (Proteaceae). Acta Hort. 185, 137- 143.

Hofer, R.M., 1995. Root hairs. In: Plant Roots, The Hidden Half. Ed. Y. Waisel, A. Eshel, and U. Kafkafi. Marcel Dekker Inc., New York NY. pp. 529-557.

Lamont, B.B., 1972. The effect of soil nutrients on the production of proteoid roots by *Hakea* species. Aust. J. Bot. 20, 27-40.

Lamont, B.B., 1982. Mechanisms for enhancing nutrient uptake in plants with particular reference to the mediterranean South Africa and western Australia. Bot. Rev. 48, 597-689.

Lamont, B.B., 1986. The significance of proteoid roots in proteas. Acta Hort. 185, 163-170.

Lamont, B., Brown, B. G. and Mitchell, D.T., 1984. Structure, environmental effects on their formation, and function of proteoid roots in *Leucadendron laureolum* (Proteaceae). New Phytol. 97, 381-390.

Marschner, H. and Romheld, V., 1995. Root-Induced changes in the availability of micronutrients in the rhizosphere. In: Plant Roots, The Hidden Half. Ed. Y. Waisel. A. Eshel, and U. Kafkafi. Marcel Dekker Inc., New York. pp. 503-528.

Racettee, S., Louis, I. and Torry, J.G. 1990. Cluster root formation by *Gymnostoma papuanum* (Casuarinaceae) in relation to aeration and mineral nutrient availability in water culture. Can. J. Bot. 68, 2564-2570.

Silber, A., Ben-Jaacov, J., Ackerman, A. and Ganmore-Neumann, R, 1996, Effect of phosphorus and Nitrogen concentration on *Leucadendron* 'Safari Sunset'.

Tiaz, L. 1984. Plant cell expansion: regulation of cell wall mechanical properties. Ann. / Rev. Plant Physiol. 35: 585-657.

Tang, C.X., Longnecker, N.E., Thomson, C.J., Greenway, H. and Robson., A.D., 1992. Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in their sensitivity to pH above 6.0. J. Plant Physiol. 6, 715-719.

White, P.F. 1990. Soil and plant factors relating to the poor growth of *Lupinus* species on fine textured alkaline soils- A review. Aust. J. Agric. Res. 41, 871-890.

Yan, F., Scubert, S. and Mengle, K., 1992. Effect of low medium pH on net proton release, root respiration, and root growth of corn (*Zea mays* L). and broad bean (*Vicia faba* L). Plant Physiol. 99, 415-521.

Table 1. Concentration of main nutrients (mg/l) in the nutrient solution.

Treatment	K	P	NO_3 mg/l	NH_4	pH
I	50	7	15	35	5.5
II	50	20	15	35	5.5
III	50	7	35	15	5.5
IV	50	7	15	35	7
V	50	20	15	35	7
VI	50	7	35	15	7

Micro-elements concentration added: Fe^{-2}, as EDDHA-Fe (sequestrine) in addition to the EDTA-based: Fe-0.69, Mn-0,34, Zn-0.17, Cu-0.025, Mo-0.019 and B-0.25 mg/l.
The nutrient solution were prepared by using commercial fertilizers and tap water consisting of approximately: NO_3-10, P-0.4, Ca-50, Mg-20, Na-100 and Cl-140 mg/l.

Table 2. Total and root fresh weight (g/plant) and percentage of root of total fresh weight of 'Safari Sunset' plants grown in an aerohydroponic system, as affected by pH (5.5-I, II, and III; 7.0-IV, V and VI), P concentration (7mg/l-I, III, IV and VI; 20 mg/l- II and V) and NH_4/NO_3 ratio 60/40- I, II, IV and V; 40/60- II and VI).

Treatment	pH	NO_3	NH_4 mg/l	P	Total fresh weight	Root fresh weight g/plant
I	5.5	15	35	7	67.2 a	21.7 a
II	5.5	15	35	20	51.5 ab	13.0 bc
III	5.5	35	15	7	58.6 ab	20.3 ab
IV	7	15	35	7	34.3 bc	7.9 c
V	7	15	35	20	23.8 c	5.7 c
VI	7	35	15	7	24.5 bc	8.4 c
F test				66***	8.12***	
$LSD_{0.05}$				20.1	7.3	

Plate 1. Effect of the pH of the nutrient solution on the development of L. 'Safari Sunset' plants, after two months in the experimental condition. A-pH 5.5, B-pH 7.0. whole plant, 2-root system.

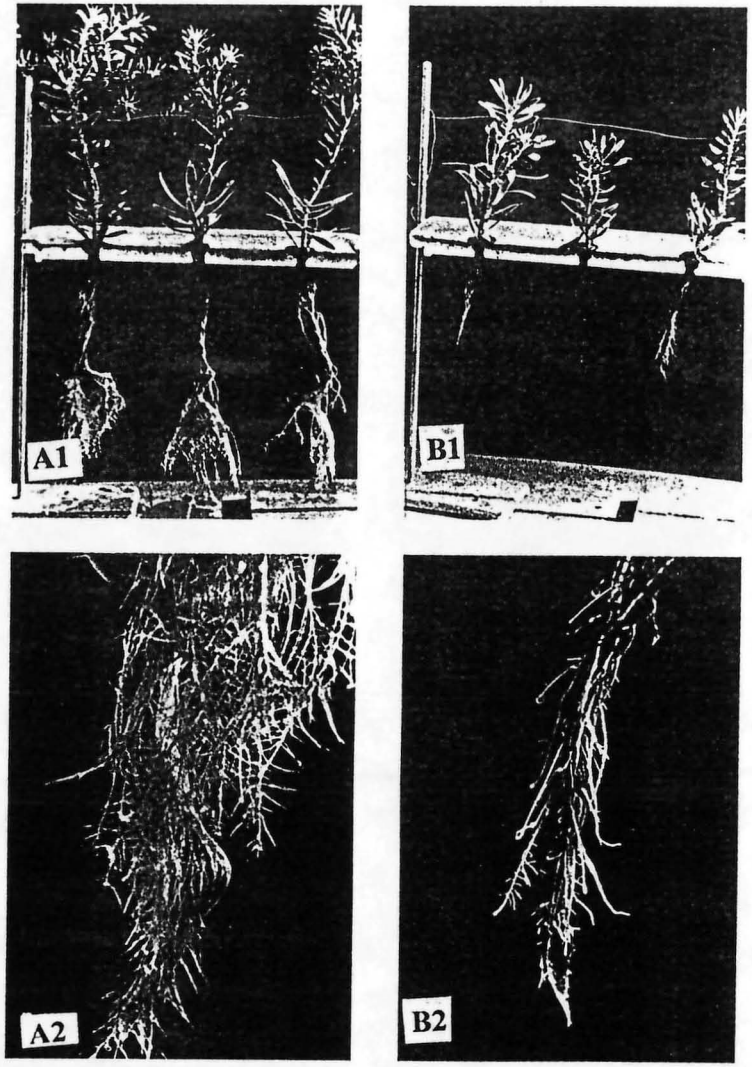
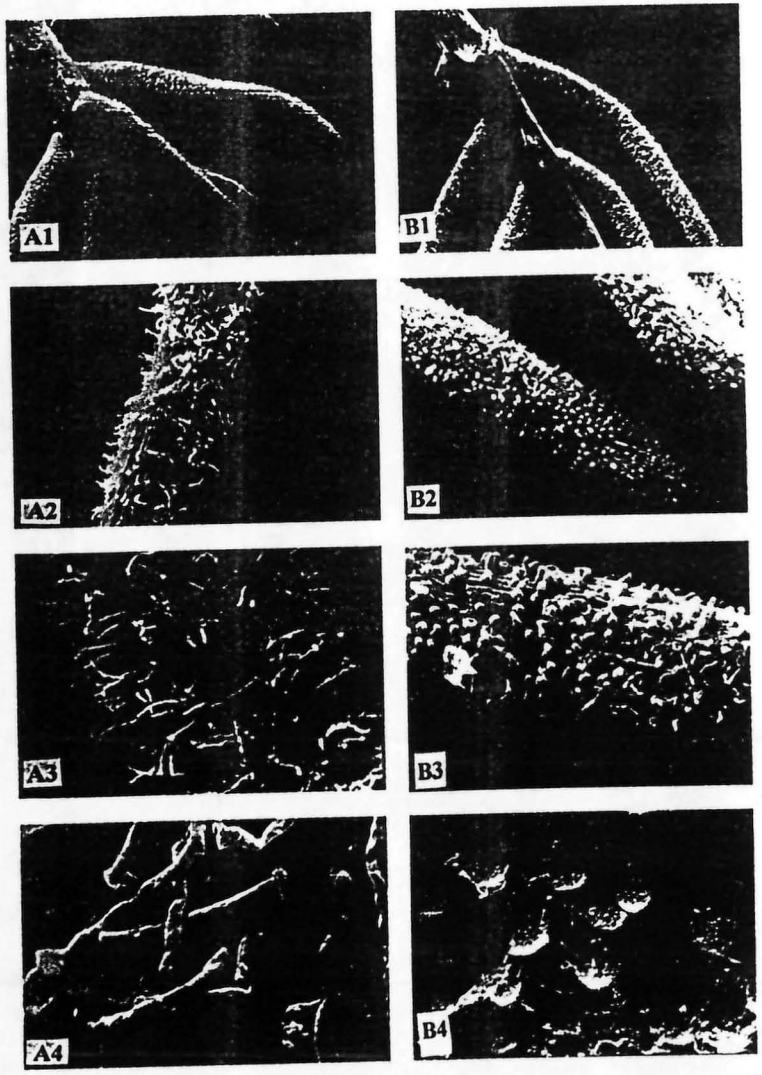


Plate 2. Scanning electron microphotographs of the effect of the nutrient solution pH on the development of root epidermal cells and root hairs of *L.* 'Safari Sunset' plants, 1.5 mm distance from the root tip. A-pH 5.5, B-pH 7.0. 1- x 35 (13 mm = 500μ), 2-x100 (8 mm = 100μ), 3-x200 (16 mm = 100μ) and 4-x1000 (8 mm = 10μ),



EVALUATION OF *LEUCADENDRON* SELECTIONS AS SINGLE STEM CU FLOWERS

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Abstract

In South Africa, the dioecious genus *Leucadendron* has largely been ignored as a source single stem cut flowers. Hybridization between species is relatively successful in this gen promising an excellent return on the investment of time and effort in breeding. Howev newly bred hybrids need to be selected and the greater the numbers to choose from, t more stringent the selection process must be. Characterization and evaluation of clon provide tools for comparing traits of importance to breeders and to commercial growe The results of characterization and yield evaluation of clones grown at Elsenbu Experimental Farm in South Africa are presented, highlighting differences betwe individual clones, as well as types of clones.

1. Introduction

The dioecious genus *Leucadendron* of the Proteaceae family has largely been ignored a cut flower product in South Africa, even though many interspecific hybrids have be produced by countries such as New Zealand (Bell, 1988). This is most likely due to t abundance of some *Leucadendron* species growing wild in South Africa and harvested fr the wild as well as to the use of broadcast sown stands for fresh and dried material. Neitl of these types of material can supply good quality, fresh, single-stem cut flowers *Leucadendron*.

Van den Berg and Brits (1995) reported on the results of preliminary hybridization a evaluation of *Leucadendron*, indicating vast potential for hybridization in comparison other genera of the Proteaceae. The ability to produce larger numbers of hybrid proge implies that greater stringency can and must be applied to the selection of worthy hyb genotypes. This enables quicker progress towards the breeding aims of high yield marketable stems per plant, long marketing period, early plant maturity and aesthe qualities including appearance, shelf and vase life.

The evaluation method reported on in this paper describes the application of evaluation system for single-stem cut flowers of *Leucadendron*, aimed at providing tools compare clones and types of hybrids, assess the genetic variability between clones a obtain an indication of the commercial potential of the clones.

2. Materials and methods

Thirty four entries in the field genebank of four different types of *Leucadendron* clon i.e. *L. laureolum* types (7 entries), *L. salignum* (15 entries), *L. salignum / L. discolor* hybri (5 entries) and *L. salignum / L. laureolum* hybrids (7 entries), were planted during Octob 1991 in a randomized block on a south west facing slope on Hutton soil. The plant spaci was 1 m in the rows, 3 m between rows. Between 10 and fifty plants of each clone we planted. Characteristics marginally affected by the environment, such as the bu characteristics, leaf shape, size and color, and flower shape and size were determined b visual inspection during 1995. These are called characterization data. Four plants of ea clone were randomly chosen for the yield evaluation. These young plants were pruned ba

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Rourke, J.P., 1972. Taxonomic studies on *Leucospermum* R.Br. Trustees of the National Botanic Gardens of South Africa, Kirstenbosch, Newlands, C.P. J. S. Afr. Bot. Supplementary volume No: 8.

Rourke, J.P., 1980. The Proteas of Southern Africa. Tafelberg, Cape Town.

Van der Merwe, P., 1985. The genetic relationhip between the South African Proteaceae. Protea News 3:3-5.

Vogts, M.M., 1980. Species and variants of Protea. Farming in South Africa Series: Flowers, Ornamental Shrubs and Trees, B.1. Department of Agriculture and Water Supply.

Vogts, M.M. & Rousseau, G.G., 1976. Propagation of proteas. Farming in South Africa Series: Flowers, Ornamental Shrubs and Trees, B.2. Department of Agriculture and Water Supply.

Von Broembsen, S.L., 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* 16:221-225.

Von Broembsen, S.L., & Brits, G.J., 1985. *Phytophthora* root rot of commercially cultivated proteas in South Africa. Plant Disease 69:211-213.

Von Broembsen, S.L. & Brits, G.J., 1989. Evaluation of the resistance of pincushion (*Leucospermum* spp.) breeding lines to root rot caused by *Phytophthora cinnamomi*. Acta Horticulturae.264

A TECHNIQUE TO IMPROVE THE PROPAGATION BY STEM CUTTINGS OF PROTEA OBTUSIFOLIA BUEK EX MEISN.

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Abstract

The rooting percentage of stem cuttings of *Protea obtusifolia* Buek ex Meisn., following the standard technique, is usually low. Longitudinal cuts made in the bark of the cutting base, permited to increase such percentage up to over 60%.

Introduction

South African proteas are normally propagated by seeds or by stem cuttings (Meynhardt, 1974; Jacobs and Steenkamp,1975 and 1976); Jacobs, 1983). *Protea obtusifolia* Buek ex Meisn. is a native species of the southern Cape Coast, where it grows in alkaline soils, with high values of pH (above 8), although it can be successfully cultivated in acid and alkaline soils (Vogts, 1982; Rourke, 1982). For this reason we have recommended it for the coastal areas of the north side of Tenerife, where banana is the main crop, which is actually suffering a bad crisis due to the entrance of the Canaries into the European Economic Community. The soils of these areas have a pH in the range of 6.5 or higher and an electrical conductivity in the range of 1.0-2.0 dS/m, or higher.

The *P. obtusifolia* plants, currently in the commercial plantations of proteas in Tenerife, have been grown from seeds imported from South Africa and for this reason there is a great variety amongst them. The plants differenciate in the colour and size of flower, the growth rate, adaptation to the ecological conditions, etc. There are plants with healthy and vigorous growth, whilst others grow with difficulty showing symptoms of chlorosis and other nutritional disorders. It is therefor necessary to make a selection of the most appropriate plants and propagate them vegetatively, thereby improving the existing plantations, obtaining a higher quality and uniformity for future plants.

The results obtained in our propagation unit, in the multiplication of *P. obtusifolia* by means of stem cuttings, following the standard technique (Jacobs and Steenkamp, 1975), were disappointing, the percentage of rooting was low. In order to improve on these results, bearing in mind that in certain woody species, such as juniper and azalea, wounding the base of the cuttings is favourable for rooting (Hartman and Kester, 1974), longitudinal cuts were made in the base of some cuttings, which resulted in root development at a much faster rate with a considerably higher percentage.

The purpose of the present study was to investigate the effects of these cuts in the rooting of cuttings from *P. obtusifolia*.

Material and methods

Terminal semi-hardwood cuttings, 15 cm long, were prepared from shoots of the current season's growth taken from numerous healthy and vigorous *P. obtusifolia* plants, approximately two and a half years old, grown in a commercial plantation in Los Realejos (Tenerife).

A randomized block design was employed with two treatments (A and B) and three replications. The experimental unit consisted of 20 cuttings. The total number of cuttings was 120.

In treatment A (control), cuttings prepared according to the standard technique were used. In treatment B, the same type of cuttings were employed, but in addition, four longitudinal cuts of 2 cm long, equally spaced, were made in the bark of their bases.

Cuttings were stripped of leaves on their basal half and a fresh cut was made at the base of each cutting, before dipping the basal 2 mm into a 50% ethanol solution of IBA (4 g/l) for five seconds, followed by a dip in "bencap" fungicidal powder (Brits, 1986). Then, they were planted in a mixture of polysterene foam pellets and peat moss (1:1 v/v) in plastic propagating trays, which were placed on a bed with bottom heat (25 $\pm$ 2°C) in a well ventilated greenhouse (polyethylene roof and plastic netting walls) at a 60% reduction of natural light. Mist irrigation was supplied by misting nozzles, at 50 l/h for 36 seconds, every 30 minutes between 09.00 and 17.00 h.

At 12, 16, 20 and 24 weeks from planting, cuttings were scored for adventitious root development. The following scale was used: 2 = transplantable; 1 = roots present but not transplantable; 0 = no roots (Brits, 1986). The results were subjected to analysis of variance and to the Duncan test.

Results and discussion

Nine weeks after the start of the experiment, it was observed that callus had appeared on the cuttings where longitudinal cuts had been made (treatment B), and some had roots or were starting to show them, whereas the majority of those used under the control treatment (treatment A) had no sign of callus formation. It proves therefor, that the cuts made are favourable in respect of the formation of callus and the consequent growth of roots.

As can be seen in figure 1, at 12 weeks from planting the overall percentage of rooting of cuttings with treatment B was 50%, against only 6.7% of those with treatment A. At 16 weeks the percentages were 55% and 11.7% respectively. This latter percentage, referring to cuttings with treatment A, was maintained throughout the experiment, whilst the percentage with treatment B increased to 58.3% at 20 weeks and 63.3% at 24 weeks, the duration of the experiment.

During the experiment the percentage of dead cuttings where treatment A was applied, increased from 0% at 12 weeks to 3.3% at 24 weeks, whilst the percentage with treatment B was 1.7% and 8.3% respectively. At the end of the experiment, the percentage of stem cuttings showing roots, but which were not transplantable was 3.3% with treatment A and 1.7% with treatment B.

At 24 weeks there was a significant difference in the overall percentages of rooting in treatment A and B, at a level of 1%. The technique used in treatment B, increased the rooting percentage to almost 5½ times more than that of treatment A.

Conclusions

The results obtained confirm that the propagation of *Protea obtusifolia* by stem cuttings, following the standard technique, can be improved upon, when prior to hormone treatment, 4 longitudinal cuts, 2 cm long, equally spaced, are made in the bark of the cutting base.

References

Brits, G.J., 1986. The influence of genotype, terminality and auxin formulation on the rooting of Leucospermum cuttings. Acta Horticulturae 185: 23-30.

Hartmann, H.T. and Kester, D.E., 1974. Compañía Editorial Continental, S.A., Mexico.

Jacobs, G. and Steenkamp, J.C., 1975. Proteas: the rooting of stem cuttings. Farming in South Africa, Series: Flowers, Ornamental Shrubs and Trees, B.3. Department of Agricultural Technical Services.

Jacobs, G. and Steenkamp, J.C., 1976. Rooting stem cuttings of Leucospermum cordifolium and some of its hybrids under mist. Farming in South Africa, Series: Flowers, Ornamental Shrubs and Trees, B.7. Department of Agricultural Technical Services.

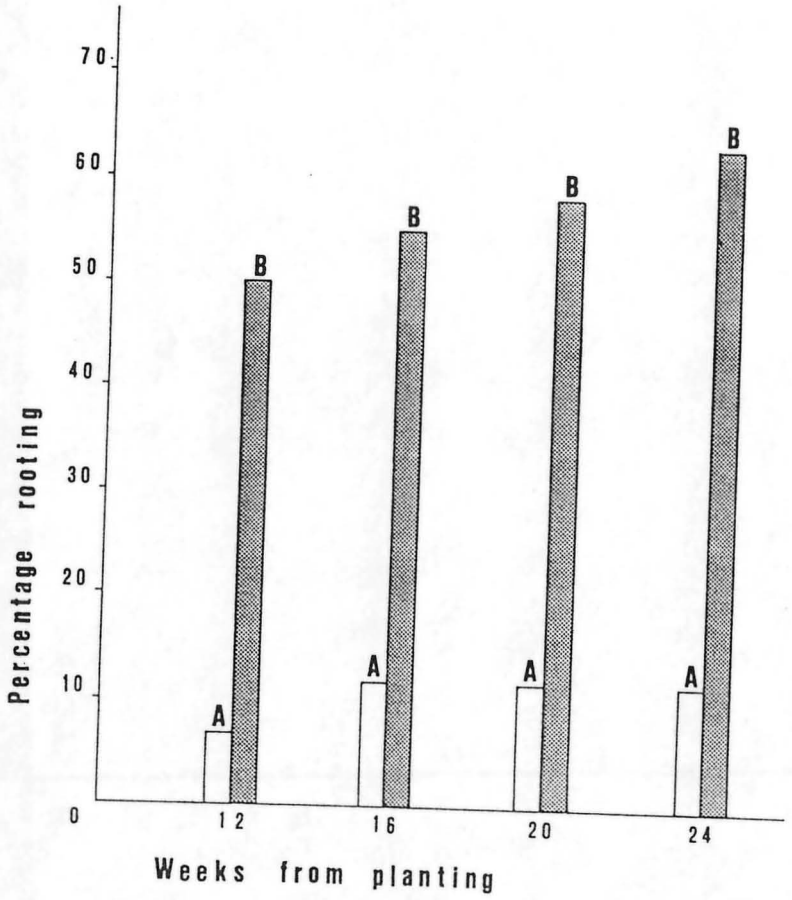
Jacobs, G., 1983. Vegetative propagation of proteas - recent developments. In Growing and Marketing of Proteas, vol. 1. Proteaflora Enterprises Pty. Ltd., Melbourne, Australia.

Meynhardt, J.T., 1974. Propagation of proteas. Farming in South Africa, Series: Flowers, Ornamental Shrubs and Trees, B.2. Department of Agricultural Technical Services.

Rourke, J.P., 1982. The proteas of southern Africa. Centaur Publishers, Johannesburg, South Africa.

Vogts, M., 1982. South Africa's Proteaceae. Know them and grow them. C. Struik, Cape Town, South Africa.

Figure 1 - Effect of basal longitudinal cuts on rooting percentage of *Protea obtusifolia* cuttings. A = control; B = cuttings with cuts.



MICROPROPAGATION OF *LEUCOSPERMUM*

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Abstract

When axillary bud explants of *Leucospermum conocarpodendron X L. cuneiforme* 'Hawaii Gold' were cultured in liquid medium of modified 1/2 MS inorganic salts, vitamins (0.25 mg/l each of pyridoxine·HCl and nicotinic acid and 0.2 mg/l thiamine·HCl), 0.2 mg/l BA and 2% sucrose, they developed proliferative bodies. When placed on filter paper bridges in shoot differentiation medium, these proliferations developed shoots which were then further elongated on agar medium. Microcuttings rooted *in vitro* were established under greenhouse conditions.

1. Introduction

Micropropagation of cut-flower protea was investigated because, in Hawaii, large quantities of vegetatively propagated plants are needed to upgrade existing fields with superior cultivars (Parvin, 1985).

Micropropagation has been successful for the following proteaceous plants: *Grevillea rosmarinifolia* (Ben-Jaacov et al., 1981), *Grevillea* hybrids (Gorst et al., 1978) and *Telopea speciosissima* (Seelye et al., 1986). Other proteas have been established successfully *in vitro* but rooting and establishment under *in vivo* conditions were not reported (Ben-Jaacov et al., 1986; Seeley et al., 1986).

2. Materials and Methods

Disinfestation procedure, initiation of culture, and culture conditions have been described previously (Kunisaki, 1989). For induction of shoot formation, proliferative bodies were placed on filter paper bridges in liquid media for shoot differentiation (Table 1) or modified 1/2 MS (Murashige et al., 1962) in which potassium and ammonium nitrates were reduced to half strength. Both media were supplemented with 0.2 mg/l benzyladenine (BA).

For root induction, microcuttings were dipped in 150 mg/l IBA solution for 10 minutes and transferred to rooting medium (Table 1) solidified with 7 g/l agar. Rooted cuttings were transplanted to potting mixture of 2 perlite:2 peat moss:1 soil and misted for high humidity.

- Magenta Sunset (*Leucadendron salignum* x *L. laureolum*)
  - excellent vigor and yield, harvestable from February to August. Developed by ARC: Fynbos Unit.
- Rosette I and II* (*Leucadendron laureolum* x *L. elimense*)
  - vigorous, erect producing long straight stems, green - February to May; yellow - June to August, green with red cone - September. Developed by ARC: Fynbos Unit.

(* Indicates plant breeders rights).

The potential and cultivation protocol of products such as *Brunia*, *Erica*, and *Phylica* are investigated and released to the industry (Rugge, personal comm.).

Basic research is undertaken on seed germination (Brits, 1995) and flower initiation (Malan, 1994). Interspecific incompatibility studies are undertaken (Van der Walt, 1995) to solve problems in developing new cultivars.

Plant protection is seen as an important problem in the industry. To determine the insect and disease component is important to develop an integrated pest management strategy (Wright and Saunderson, 1995). If the protection programme is successful it will allow farmers in the position to deliver environmentally friendly products to the market.

5. Conclusion

If the indigenous flower industry uses its opportunities and addresses its threats, the industry has the potential to keep and increase its market share. This industry growth will expand the essential job opportunities needed for women in South Africa. The export product that can be produced won't compete with producers in Europe, as Europe cannot cultivate fynbos on a large scale. With the correct strategic approach, the fynbos industry can accommodate new farmers in floriculture and informal pickers from the wild can become part of the formal agricultural sector.

Acknowledgments

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References

Brits, G.J., Cutting, J.G.M., Brown, N.A.C. and Van Staden, J., 1995. Environmental and hormonal regulation of seed dormancy and germination in Cape fynbos *Leucospermum* R. Br. (Proteaceae) species. A working model. Plant Growth Regulation 17: 181-193.

Coetzee, J.H. and Brits, G.J. 1991. Review of floricultural research in South Africa, with special emphasis on the protea cultivars released by the Fynbos Research Unit. Conference proceedings, IPA 6th Biennial Conference, Australia.

Coetzee, J.H. and Littlejohn, G.M., 1995. Ornamental horticulture in rural areas in South Africa. Acta Hort. 391: 173-180.

Malan, D.G., Cutting, J.G.M. and Jacobs, G. 1994. Inflorescence development in *Leucospermum* "Red Sunset": Effect of Benzyladenine and changes in endogenous cytokinin concentrations. J. S. A. Soc. Hort. Sci. 4: 37-41.

Van der Walt, I.D. 1995. Pollen Biology in relation to artificial hybridization in the genus *Protea*. M.Sc. thesis, University of Stellenbosch, Stellenbosch.

Wright, M. G. and Saunderson, M.D., 1995. Protea plant protection: from the African context to the international arena. Acta Hort. 387: 129-137.

IMPROVED METHODS FOR ROOTING CUTTINGS OF *PROTEA OBTUSIFOLIA*

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Abstract

Methods for improved vegetative propagation of *Protea obtusifolia* were examined. Different hormone treatments, seasons, mother plant treatments, and cuttings in different physiological age were tested. The results showed that the use of semi hardwood cuttings and treating them with IBA 2000 ppm gave the highest rooting percentage. Growing mother plants under 30-50% shading net improved rooting percentage. Rooting of cuttings was improved greatly by repeated rooting for 3 generations. Cuttings taken from the last generation of plants rooted at 75% compared to 37% when cuttings were taken from matured field grown plants.
Using these methods, we got high rooting percentage of 75% in a period of 10 weeks.

1. Introduction

It is difficult to grow Proteas in Israel. The climate, the soil and the water are not ideal for successful commercial production of Proteas as cut flowers. *Protea obtusifolia* is one of the species which is very tolerant to alkaline soil and grows in limestone soils in nature. This fact makes it so important for the introduction of Proteas as cut flowers in Israel (Ben-Jaacov 1986). *Protea obtusifolia* is compatible graft on many other Protea species, thus, it can be used as a rootstock for those species (Brits 1990a, Brits 1990b, Maffott and Turnbull 1994). In the last 20 years there have been several attempts to grow *Protea obtusifolia* in Israel, but most of the plants were raised from seeds, showed high genetic variability and therefore were of low commercial value. The highest rooting percentage of *P. obtusifolia* obtained by Perez (1992) was 10% after 40 weeks. In order to propagate *P. obtusifolia* as cut flowers or as rootstocks it is important to improve vegetative propagation.

In this study we tested several methods, the most successful are presented in this paper.

2. Material and methods

2.1. Plant material

The first generation of cuttings were collected from 8 years old clonal mother plants grown at the Volcani Center at Bet Dagan. All plants were grown under the same environmental conditions unless it was the factor investigated.

2.2. Growth regulators

Cuttings were treated with IBA as powder or liquid, and with an experimental auxin conjugate.

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2.3. Rooting conditions

Cuttings were placed in rooting tables heated in winter to 28°C in medium of coconut fibers; polystyrene 1:1 (V:V), in a greenhouse equipped with evaporative cooling system.

Rooting was done under mist conditions of 10-20 seconds every 10-15 minutes. The mist conditions were changed according to the climatic conditions.

3. Results

3.1. Growth regulators

After few experiments with different concentrations of growth regulators we found that IBA as powder in concentration of 0.4%, and as liquid at 2000 ppm gave the highest rooting percentage (Table 1). IBA at 300 ppm (data not shown) and the auxin conjugate produced poor results.

3.2. Cutting's age and physiological condition.

3.2.1. Cuttings in 3 growth stages were tested:

Herbaceous, semi hardwood and hardwood. The semi hardwood cuttings rooted at the highest percentage (Table 2).

3.2.2. Cuttings were taken from mother plants in two physiological ages:

Juvenile (one year old) and mature (8 years old). Cuttings that were taken from the juvenile plants rooted at higher percentage (Table 3).

3.2.3. Cuttings were taken from 3 generations of *P. obtusifolia* plants.

Generation II was raised vegetatively from cuttings taken from generation I, and generation III plants were raised from cuttings taken from generation II. Cuttings from generations II and III (Table 4) rooted at higher percentage than those taken from generation I. The difference between generation II and I was greater than the difference between generation II and III.

3.3. Mother plants treatment

Mother plants with actively growing shoots were covered with shading nets of 30% or 50% shade.

The cuttings from shaded plants rooted at a higher percentage than sun grown plants (Table 5). Cuttings grown under 50% shade showed higher percentage of rooting than cuttings produced under 30% shade, but both rooted better than the control unshaded cuttings.

3.4. Rooting season

Cuttings from 2 clones of *P. obutsifolia* were taken during the year, on the 15th of every month. The results (Table 6) show that in the hot season (in Israel), June-October, the rooting percentage was low. Rooting was improved in the fall and winter and the best rooting was achieved in the spring (April).

4. Discussion

This study demonstrated that several factors influence rooting: proper treatments of the stock plants and the cuttings lead to rapid rooting with high percentage of success.

1. Mother plants: First generation of cuttings taken from matured (8 years old) plants rooted poorly (40%). Cuttings taken from the second generation of mother plants, grown under partial shade (best at 50% shade) rooted better and faster. Cuttings taken from the third generation rooted best and fastest (80% in 10 weeks).
2. Best time for sticking the cuttings was in the spring and the best hormonal treatment was a ten second dip of the bases of the cuttings in 2000 ppm IBA.

Table 1. Influence of growth regulators as powder or as liquid in various concentrations, on rooting of *P. obtusifolia* cuttings.

Treatment	Control	IBA (Powder)		IBA (Liquid)		Auxin Conjugate
		0.8%	0.4%	4000ppm	2000ppm	(Powder) 0.2%
Rooting %	30 c*	43 b	66 a	48 b	72 a	8 d

* Different letters represent significant difference between treatments.

Table 2. Influence of growth stage of the plant material on the rooting of *P. obtusifolia* cuttings. The cuttings were treated with IBA 4000 ppm.

Cuttings = growth stage	Rooting %
Herbaceous	5 c*
Semi-hardwood	78 a
Hardwood	43 b

* Different letters represent significant difference between treatments.

Table 3. Influence of the physiological age on rooting of *P. obtusifolia* cuttings. The cuttings were treated with IBA 2000 ppm.

Physiological age	Rooting %
Juvenile (1 year old)	60 a*
Mature (8 years old)	
"Red"	13 b
"Pink"	10 b

* Different letters represent significant difference between treatments.

Table 4: Rooting of cuttings from 2 clones of *P. obtusifolia* and Pink Ice taken from 3 consecutive generations. The cuttings were treated with IBA 2000 ppm. Data collected 10 weeks after the cuttings were stuck.

Plant	Rooting %		
	Generation I	Generation II	Generation III
Pink Ice	43.2 b*	75.0 ab	87.5 a
Clone C-82	37.5 b	68.7 ab	81.2 a
Clone LK-1	37.5 b	62.5 ab	75.0 a

* Different letters represent significant difference between treatments.

Table 5: Rooting of *P. obtusifolia* cuttings taken from mother plants grown under 30% or 50% shading nets, after 6 and 10 weeks. all cuttings were treated with IBA 2000 ppm.

Treatment of mother plants	Rooting % after	
	6 weeks	10 weeks
30% shading	40	70
50% shading	55	80
Control non-shaded	10	30

Table 6: Influence of the season on rooting of *P. obtusifolia* cuttings. Cuttings were treated with IBA 2000 ppm. Cuttings were taken on 15th of every month.

	Rooting % Clone	
Month	LK-1	C-82
Jan	60	58
Feb	70	70
Mar	68	78
Apr	78	80
May	58	63
Jun	40	49
Jul	28	25
Aug	23	20
Sep	25	28
Oct	38	40
Nov	55	55
Dec	60	63

References

Brits, G.J. (1990a). Rootstock Production Research in *Leucospermum* and *Protea*: I. Techniques. Acta Hort.264: 9-25.

Brits, G.J. (1990b). Rootstock Production Research in *Leucospermum* and *Protea*: II. Gene Sources. Acta Hort. 264: 27-40.

Maffott Judit and Turbull Lois. (1994). Grafting Proteas. Published and available from Judit Maffott. Nanju Protea Nursery Mail Center, 582 Toowoomba Queensland, 4352, Australia.

Ben-Jaacov, J. (1986). Protea Production in Israel. Acta Hort. 185: 101-110.

Perez, J.A.R. (1992). Propagation by leaf bud cuttings of *Leucadendron* 'Safari Sunset' *Leucospermum cordifolium*, *Leucospermum Patersonii* and *Protea obtusifolia*. Acta Hort. 316: 35-45.

INTRODUCTION OF PROTEAS FOR CUT FLOWER AND FOLIAGE IN TENERIFE

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Abstract

During the last six years, most of the Australian and South African proteas species cultivated for cut flower and foliage, have been planted in several experimental plots located in the north side of Tenerife, between 100 and 750 m above sea level. The best results have been obtained at heights of 500-750 m a.s.l., where environmental conditions seem to be optimum for protea cultivation. Among the species which are being cultivated successfully are: *Banksia ashbyi, B. prionotes, B. speciosa, Leucadendron coniferum, L. laureolum, L. meridianum, Leucospermum cordifolium, Protea cynaroides, P. eximia, P. grandiceps, P. longifolia, P. neriifolia and P. obtusifolia.*

Introduction

The need to find alternative crops in order to improve the profits of the farms located in the zones called "de medianías" of Tenerife, between 400 and 800 m of altitude above sea level, and to substitute partialy banana plantations, whose profits had gradually decreased during the last years, move us to introduce protea cultivation in the island, as in other islands of similar volcanic origin (Maui, Madeira), they were being cultivated successfully.

Field trials

In 1982, the first species were planted in the experimental plots of the Aclimatization Garden (Puerto de la Cruz) and in a small farm in Redondo (Icod de los Vinos), at 100 and 750 m a.s.l., respectively, in order to observe proteas behaviour in the subtropical coastal area, occupied mainly by banana plantations, and in the "medianías" area with a mediterranean climate and with crops of potatoes, corn, apples, plums, etc. The Garden soil had a pH of 5.9-6.9 and an electrical conductivity (EC) of 0.7-1.0 dS/m, while in Redondo, the soil had a pH of 5.5 and an EC of 0.3 dS/m. During the following years, we could see that the plants grew better in Redondo than in the Garden and that some species did not tolerate environmental coastal conditions. The species that, up to now, have grown well in the Garden are: *Banksia ashbyi, Leucadendron argenteum, L. coniferum, L. linifolium tortum, L. meridianum, L. nobile, Leucospermum cordifolium, Lsp. reflexum, Lsp. praecox and Protea obtusifolia.* In Redondo: *Leucadendron laureolum, Leucospermum cordifolium, Lsp. reflexum, Protea burchellii, P. compacta, P. cynaroides and P. laurifolia.*

During 1985 and 1986, Australian and South African species were planted in Palo Blanco (Los Realejos), in the fields of a school, at about 650 m a.s.l. The soil was acid (pH=5.0 - 5.5) and had a low EC (0.4 - 0.6 dS/m). Nearly all the species (about 30) are perfectly growing and some of them have already bloomed, the flowers having been sold in the local market. Among the cultivated species are:

Banksia ashbyi, B. occidentalis, B. prionotes, B. speciosa, Leucospermum cordifolium, Protea cynaroides, P. eximia, P. grandiceps, P. longifolia, P. neriifolia, P. obtusifolia and *Telopea speciosissima*. The cultivated area is of 0.25 acres.

In the end of 1986 a new experimental plot of about 1 acre was established in Valle Guerra (La Laguna) at 150 m a.s.l. The soil had a pH of 6.0 - 6.5 and a EC of 1.5 - 2.0 dS/m. More than 30 species and cultivars of Southafrican and Australian proteas had been planted, but most of them have not tolerated the environmental conditions of the place, having suffered chlorosis and other nutritional disorders. *Banksia integrifolia, Leucadendron meridianum, L.'Safari Sunset', L. uliginosum ssp. uliginosum, Leucospermum cordifolium, Lsp. cuneiforme* and *Lsp. patersonii* are growing well or showing some symptoms of chlorosis.

In 1987, 26 Southafrican and Australian species were planted in La Florida (La Orotava), at 520 m a.s.l. The pH of the soil was about 7 and the EC=0.3 - 0.4 dS/m. Most of the species are growing well and the following have already bloomed: *Banksia prionotes, B. speciosa, Leucospermum cordifolium, Protea eximia, P. longifolia, P. neriifolia* and *Serruria florida*. The cultivated area is of 1.5 acres.

Florican, an important co-operative of flower growers offered in the beginning of 1988, a plot in Los Rodeos (La Laguna), at 600 m a.s.l. for trials on proteas. The soil had a pH of about 5 and an EC of 0.7 dS/m. Up to now, 27 Australian and Southafrican species and cultivars had been planted. Most of them are growing well.

All the plants in the experimental plots have been obtained from seeds imported from South Africa and Australia, with the exeption of *Leucadendron 'Safari Sunset', Leucospermum cordifolium 'Riverlea', Lsp. 'Harry Chittick'* and *Lsp. patersonii*, which were imported from New Zealand as cuttings and rooted in our propagation unit that is working since the end of 1986.

Conclusions

The results obtained up to now show that proteas can be cultivated in the north side of Tenerife, being the best places those located between 500 and 750 m above sea level.

References

Parvin, P.E., Criley, R.A. and Bullock, R.M. 1973. Proteas: developmental research for a new cut flower crop. Hortscience 8 (4):299-303

Vogts, M. 1982. Proteaceae - know them and grow them. C. Struik, Cape Town.

Watson, D.P. and Parvin, P.E. 1970. Culture of ornamental proteas. Hawaii Agricultural Experiment Station. Research Bulletin 147: 1-24.

Watson, D.P. and Parvin, P.E. 1973. Ornamental Proteas - New Cut Flower Crop. Hortscience 8(4):290.

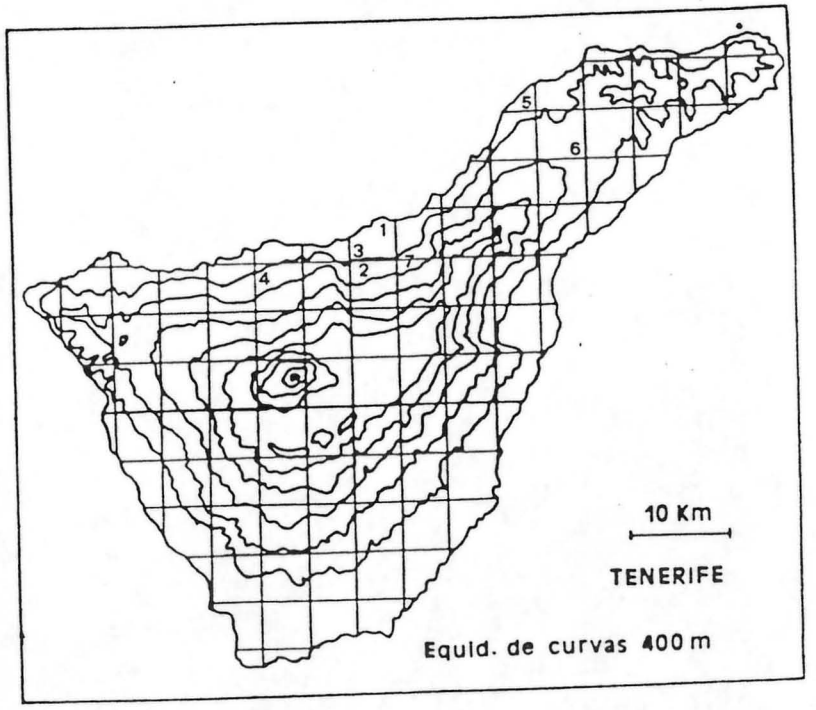

Figure 1. Distribution of protea cultivation in Tenerife: 1.Puerto de la Cruz; 2.Palo Blanco (Los Realejos); 3.Azadilla Alta (Los Realejos); 4.Redondo (Icod); 5.Valle Guerra (La Laguna); 6.Los Rodeos (La Laguna); 7.La Florida (La Orotava).

should be held in high light conditions, if possible. The fact that blackening is substantially accelerated in the dark indicates a means by which growers or breeders could select cultivars of species such as *P. eximia* and *P. neriifolia* that are less affected by the problem. Many seedlings could easily and rapidly be screened by placing their cut flowers in a warm, dark environment for a few days. Lastly, the possibility that leaf blackening could be delayed by harvesting flowers in the afternoon, when their carbohydrate status is greatest, warrants examination.

5. References

Brink, J.A., and de Swardt, G.H., 1986. The effect of sucrose in the vase solution on leaf browning of *Protea neriifolia* R. Br. Acta Hortic. 185:111-119.

Ferreira, D.I., 1983. Prevention of browning of leaves of *Protea neriifolia* R. Br. Acta Hortic. 138:273-276.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. inflorescences. Acta Hortic. 185:121-129.

Halevy, A.H., Mayak, S. (1979) Senescence and postharvest physiology of cut flowers, Part 1. In: Horticultural Reviews, Vol. 1, pp. 204-236, Janick, J., ed. AVI, Westport.

Paull, R., Criley, R.A., Parvin, P.E., and Goo, T. (1980) Leaf blackening in cut *Protea eximia;* importance of water relations. Acta Hortic. 113:159-166.

Reid, M.S., van Doorn, W., and Newman, J. (1989) Leaf blackening in Proteas. Acta Hortic. *In press.*

Whitehead, C.S., and de Swardt, G.H., 1982. Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of Protea neriifolia. South African J. Bot. 1:127-130.

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POST-HARVEST WATER RELATIONS OF LEUCADENDRON CV. SILVAN RED

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Abstract

Vase life of mature stems of Leucadendron cv. Silvan Red (Ld. laureolum x Ld. salignum) fell from six to four and a half weeks, after exposure to a transient water stress, in the form of strong drying conditions for 30 hr, during which leaf water potentials fell to -3200 kPa.

Vase life was identified by the appearance of localised desiccation of leaves, starting from the tips and extending towards the leaf-base. Ion leakage from the leaves, as a whole, was not detected, but localised leakage of solutes within leaves may have occurred and not been detected. Leaf water relations, were similar for both treatments, with turgor declining from 1300 kPa, initially, to zero by about day 37, and solute potential increasing from -1450 kPa, initially, to -1150 kPa by day 37, indicating loss of solutes. The latter was consistent with loss of dry weight. Transpiration of stressed stems fluctuated in the experiment but fell to a level, similar to the controls and after 45 days had fallen steadily to one fifth of the initial value. Leaf turgor in cv. Silvan Red can be expected to respond to various treatments e.g. pulsing solutions, to improve food supply, but it is not known whether such treatments will alleviate the effects of severe transient stresses on vase life.

1. Introduction

Vase life of cut flowers depends on cell turgor, i.e. plant cell water relations. Yet, measurements of plant water relations are seldom reported in studies of post-harvest treatments designed to prolong vase-life of flowers. In the present study we studied the effect of a water stress on Leucadendron cv. Silvan Red, which is widely grown as a commercial cultivar in Australia; cv. Silvan Red was chosen for its reputedly long vase life.

2. Methods and Materials

2.1. Conditions. Mature stems (80) of cv. Silvan Red were harvested to a length of about 40 cm, held in de-ionised boiled water and taken to the laboratory. Lower parts of stems were then stripped of leaves, recut under water, and put in lots of nine stems per 1 litre plastic beaker, into a weak bromine solution (0.01 g available bromine per litre), for the experiment. To minimise plugging due to micro-organisms, 1 cm of stem was cut off every 5 days and vase solution changed every 2 days.

Stems were exposed to a constant temperature of 20 ^{o}C, and to light from a bank of Sylvania 'GROW-LUX' fluorescent tubes, which provided, over a 12 hr period each day, 150 micro-Einsteins m^{-2} s^{-1} of radiation (measured at half-stem height), which was predominantly in the blue and red bands of the spectrum.

2.2. Water stress treatment. On the day of harvesting, one half the number of stems was taken out of water and exposed to a forced draught of air under continuous light for 30 hr. At the end of this period leaf water potentials varied between -2800 and -3200 kPa. By day 25, no evidence of stress was visible, and so the stress treatment was repeated.

2.3. Measurements. Ion leakage across the plasmalemma was measured on single leaves, 4-8 cm below the involucral bracts, initially every 4 days until day 8, and thereafter every 8 days. The method involved cutting leaves into thin slices, and used a standardized procedure, involving successive rinsings. Finally, the preparation was incubated with gentle agitation for 130 minutes, rinsed, and electrical conductivity of the solution measured after 15 minutes with a PT-18 ACTIVON digital conductivity meter. This procedure was designed to confine ion leakage, during measurement, to ions originating from the symplast. This was indicated by a steady state conductivity response, which followed an initial rapid increase in conductivity of the osmoticum, interpreted as resulting from an initial flush of readily diffusing ions from the apoplast. Leakage was expressed as a percentage of the total conductivity, determined as above, on solution left to equilibrate for 48 hr at 20 °C, after cell disruption by two cycles of snap freezing in liquid N.

Leaf water potential (ψ_l), solute potential (ψ_s) and relative water content (RWC) were determined at 4-7 day intervals, on leaves (6 replicates) taken from 4-8 cm below the involucre, prior to the onset of the light cycle. ψ_l was determined by pressure bomb (Scholander *et al* , (1965), ψ_s, on expressed sap, by thermocouple psychrometer, and RWC by the method of Barrs (1968). Leaf turgor was calculated using the relationship $\psi_l = \psi_s + \psi_t$, where ψ_t is the leaf turgor potential and usually takes positive values; ψ_s, the solute potential is always negative and has the same value, but opposite sign to the osmotic pressure of the cell sap.

Transpiration, absorption of water, and fresh weight were determined from records of weight changes of vase solution and single stems (4 replicates) sealed into a conical flask and exposed to the same conditions as for the main experiment. Leaf diffusion conductances were determined (10 replicates) by porometry at mid-day every 4- 8 days on the abaxial side of leaves 4-8 cm below the involucre. Bracts on treatment stems exhibited signs of desiccation after the second stress cycle and so it was decided to repeat measurements being made on lower leaves on the bract tissue for both treatments on days 37 and 46. Drying rates of initially turgid, detached leaves and bracts (4 replicates) of both treatments, were determined under prevailing light conditions, at 0.5 to 2 hour intervals over a 12 hour period at the beginning and the end of the experiment.

Pressure-volume (PV) curves were determined on leaves (4-6 cm below the involucre) of freshly harvested stems taken through a drying cycle, using the method of Wilson *et al* (1979), excepting that holding times were greater, varying from 20 minutes, for pressures <1500 kPa, to 30 minutes for pressures >1500 kPa. Leaves were wrapped in plastic film and left overnight in water to become fully turgid. Only leaves with an initial balance pressure of <100 kPa were used and their fresh weight taken as the fully turgid weight for RWC determinations. The inverse balance pressure was plotted against corresponding RWC and the data from 4 replicates pooled for curve fitting. Wilting point was estimated as the negative of the balance pressure at the intersection of curves fitted to the linear and curvilinear parts of the plot.

3. Results

At day 27 no physical differences in the stems could be detected between treatments. By day 15 up to 60 per cent of stems in both treatments had initiated two small lateral shoots arising from the below the involucre. By day 46 these had trebled in size.

During the second stress cycle starting at day 27, 15 per cent of stressed stems had symptoms of irreversible desiccation localized at the tips of the bract leaves. Up to 40 per cent of the bracts from each stem were affected; drying progressed from the tip downwards, for a distance of about 5 mm. This condition worsened as the stems aged. By day 46, 77 per cent of the stressed stems showed varying levels of dessication (Table 1). The severity of the desiccation varied from 3-4 bracts affected per stem to all the bracts being totally dry and the involucre dead. By contrast only 8 per cent of the control stems had bracts, which were beginning to dry out. In both treatments dessication was confined to the bract or the cone.

Table 1 - Stem condition at the end of the experiment.

	Number of stems with symptoms of aging	
Observation	Control	Stressed
Cone dead or dying	0	5
Bracts drying	4	15
Bract and lower leaves drying	0	1
Cone and bracts drying	0	7
Totally dry	0	3
Lateral shoots	28	25

Ion leakage stayed essentially constant, for bracts and leaves of the two treatments, throughout the experiment, at approximately 7 per cent of total conductivity.

Stomata stayed open during the light period as indicated by leaf conductances, which were similar for both treatments. Also drying curves for leaves and bracts, under standard drying conditions, were the same after 46 days. These data did not correlate at all with transpiration, which in the control, declined steadily over the 45 day period to one fifth of the initial value. Stressed stems' transpiration rate was initially 40 per cent of the control rising to 100 per cent at day 14 and thereafter declining rapidly over a 10 day period to reach a similar level to the controls. The initial rapid rise in water usage in the stressed stems, was not observed after the second stress cycle.

Absorption of water was essentially the same as transpiration for both treatments throughout the experiment , but this was not reflected in stem fresh weights, which stayed fairly constant at about 100 per cent of initial fresh weight until day 24, but thereafter declined steadily to 94 per cent in both treatments. Weight of dry matter also declined, by about 14 per cent in both treatments, indicating a net loss of water and of solutes.

Leaf water relations, as indicated by water potentials, were similar for both treatments but varied over the experiment (Figure 1). Turgor was estimated by difference between the ψ_l and ψ_s and declined from 1300 kPa, initially, to zero by about day 37. Solute potential increased from -1450 kPa initially to -1150 kPa. The rise in ψ_s is interpreted as a loss of solutes, and is consistent with the decline in dry matter referred to above. Since

the decline in ψ_l was much greater than could be accounted for by a loss of solutes, it represents an increasing water deficit in the leaves. This was probably due to increasing hydraulic resistance, in the stem conducting vessels, which may have accompanied shrinkage of stem tissue as its water content declined. Since ψ_s was determined at the end of the dark period, the evidence of a water deficit also indicates that transpiration was taking place during the dark period, i.e. that the stomata were staying open.

The critical leaf water potential (ψ_{crit}) (Cowan, 1965), i.e. the value of ψ_l when turgor pressure (ψ_t) = 0, at which pronounced wilting occurs, was estimated from the plot of $1/\psi_l$ v. RWC (Figure 2), as approximately -18 kPa. This value relates to fresh unstressed material at the start of the experiment and compares with a ψ_l of approximately -30 kPa, in the stressed material, also at the beginning of the experiment.

4. Discussion

Vase life of c.v. Silvan Red was of the order of 6 weeks. The experiment failed to detect any general loss of membrane integrity as would be indicated by solute leakage from cells. However the imposed water stress reduced vase life by at least 10 days, the end of which was identified by the appearance of localised desiccation of leaves, starting from the tips and extending towards the leaf base. These observations appear to be similar in kind, and probably have a similar origin to the phenomenon of leaf browning commonly observed in *Protea* spp. (eg. Ferreira, 1986). However they are far milder in intensity and this may be due to a number of factors, including differences in the relative sizes of assimilate sinks and sources in the flower head, and leaves and bracts, respectively, as well as in the abundance of specific compounds, eg. phenolics, in the leaves.

The experiment indicates that when bacterial plugging of xylem vessels is not a serious factor limiting the water supply, vase life of cv. Silvan Red will tend to be correlated with a decline in leaf and bract turgor, caused by reduced osmotic pressure of the cell sap. The data also show that a severe transient water stress may still over-ride, and shorten, any slower, longer term decline, and as a result, reduce the vase-life of the stressed stems.

References

Barrs, H.D., 1968. Determination of water deficits in plant tissues. In "Water deficits and plant growth" Vol 1. (Ed T.T. Kozlowski). Academic Press, London.

Brink, J.A., and G.H. de Swardt., 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R Br Acta Hort. 185: 111-119.

Cowan, I.R., 1965. Transport of water in the soil-plant-atmosphere system. J. Appl. Ecol. 2: 221-239.

Ferreira, D.I., 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R Br. inflorescences. Acta Hort. 185: 121-130.

Scholander, P.F., Hammel, H.T., Bradstreet, D., and Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science, N.Y. 148: 339-346.

Wilson, J.R., Fisher, M.J., Schulze, E.D., Dolly, G.R., and Ludlow, M.M , 1979. Oecologia 41, 77

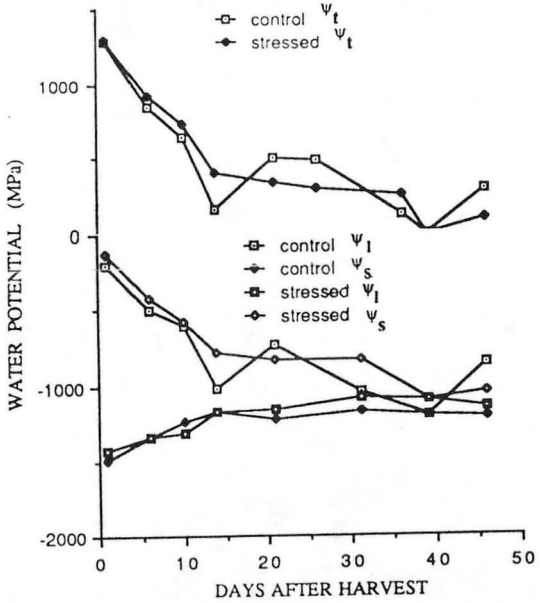

Figure 1 - Changes in leaf water potential (ψ_l) and components, the leaf sap solute potential (ψ_s) and the turgor potential (ψ_t). Each point is the mean value of four leaves from 4 - 6 cm below the involucre.

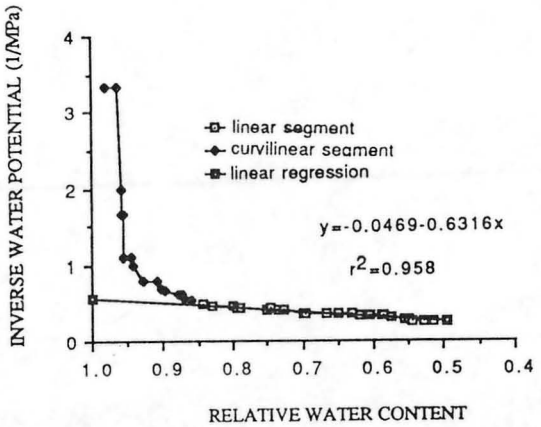

Figure 2 - Pressure-volume curve for Leucadendron cv. Silvan Red leaves (4) taken from 4 -6 cm below the involucre on freshly harvested stems.

throughout the ripening period, and therefore establish any flaws in the gas sampling techniques.

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References

Burg, S.P. and Burg, E.A., 1965. Gas exchange in fruits. Physiol. Plant., 18: 870–883.

Davies, J.N. and Maw, G.A., 1972. Metabolism of citric and malic acids during ripening of tomato fruit. J. Sci. Food Agric., 23: 969–976.

Grange, R.I. and Andrews, J., 1993. Growth rates of glasshouse tomato fruit in relation to final size. J. Hortic. Sci., 68: 747–754.

Grange, R.I. and Andrews, J., 1995. Respiration and growth of tomato fruit. Plant Cell Environ. (in press).

Kader, A.A., Zagory, D. and Kerbel, E.L., 1989. Modified atmosphere packaging of fruit and vegetables. Crit. Rev. Food Sci. Tech., 28: 1–30.

Lyons, J.M. and Pratt, H.K., 1964. Effects of stage of maturity and ethylene treatment on respiration and ripening of tomato fruits. Proc. Am. Soc. Hortic. Sci., 84: 491–500.

McGlasson, W.B., Dostal, H.C. and Tigchelaar, E.C., 1974. Comparison of propylene-induced responses of immature fruit of normal and rin mutant tomatoes. Plant Physiol., 55: 218–222.

Ng, T.J. and Tigchelaar, E.C., 1977. Action of the non-ripening (nor) mutant on fruit ripening of tomato. J. Am. Soc. Hortic. Sci., 102(4): 504–509.

Patching, C.R., Maw, G.A. and Davies, J.N., 1975. Metabolism of glucose during ripening of detached tomato fruit. J. Sci. Food Agric., 26: 23–29.

Pearce, B.D., Grange, R.I. and Hardwick, K., 1993. The growth of young tomato fruit. I. Effects of temperature and irradiance on fruit grown in controlled environments. J. Hortic. Sci., 68: 1–11.

Saltveit, M.E. Jr., 1993. Internal carbon dioxide and ethylene levels in ripening tomato fruit attached to or detached from the plant. Physiol. Plant., 89: 204–210.

Sawamura, M., Knegt, E. and Bruinsma, J., 1978. Levels of endogenous ethylene, carbon dioxide, and soluble pectin, and activities of pectin methylesterase and polygalacturonase in ripening tomato fruits. Plant Cell Physiol., 19: 1061–1069.

Šesták, Z., Čatský, J. and Jarvis, P.J., 1971. Plant Photosynthetic Production. Manual of Methods. Dr. W. Junk Publishers N.V., The Hague, pp. 132–134, 169, 289.

Shellie, K.C. and Saltveit, M.E., 1993. The lack of a respiration rise in Muskmelon fruit ripening on the plant challenges the definition of climacteric behaviour. J. Exp. Bot., 44: 1403–1406.

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Sucrose prevents foliage desiccation in cut *Leucadendron* 'Silvan Red' during cool storage

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Abstract

Pulsing *Leucadendron* 'Silvan Red' stems with sucrose solutions of 200 g l^{-1} (20%) or higher for 24 h at 1°C prevented leaf desiccation during 42 days dry storage at 1°C. Stems pulsed with sucrose for 24 h at 20°C absorbed significantly more sucrose than stems pulsed at 1°C. Despite this, leaf desiccation was significantly higher in stems pulsed at 20°C, indicating a possible toxic effect due to an oversupply of sucrose. Fresh weight was not affected by sucrose pulse concentration before or after storage. Sorbitol and mannitol pulses also had no effect on flower fresh weight, but accelerated leaf desiccation, suggesting that the inhibition of leaf desiccation by sucrose was not a result of improved leaf hydration. Total soluble sugar content in leaf tissue declined during storage; this decline was significantly inhibited by a ≥20% pre-storage sucrose pulse. A 24 h pulse with ^{14}C-sucrose indicated that sucrose was distributed primarily to the leaves. It is possible that exogenous sucrose prevented leaf desiccation in *Leucadendron* by maintaining membrane integrity.

Keywords: Leaf desiccation; *Leucadendron*; Sucrose; Cool storage

1. Introduction

Pre-storage sucrose pulsing is known to increase the subsequent longevity of cut flowers (Halevy and Mayak, 1981) by improving the osmotic concentration of flower petals (Acock and Nichols, 1979) and replacing depleted carbohydrates (Halevy, 1976). Sucrose was also thought to enhance the storage performance of cut flowers by replacing carbohydrates depleted during storage at temperatures above 0°C (Goszczynska and Rudnicki, 1988). Sucrose can also act as a protectant against damage during freezing, as applied sucrose protected carnation petals from tissue damage during five days storage at −4°C (Heins et al., 1981). Most studies in this

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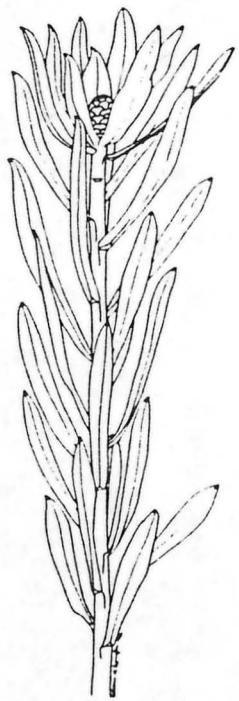

Fig. 1. Morphology of *Leucadendron* 'Silvan Red', showing the terminal cone-shaped flower, surrounded by leaves.

area have concentrated on cut flowers and petal tissue (Goszczynska and Rudnicki, 1988), and little is known of the possible role of sucrose in reducing storage damage in foliage tissue.

The hybrid *Leucadendron* 'Silvan Red' is grown extensively in south-eastern Australia and has become a major export crop. The genus *Leucadendron* is dioecious, with the small male and female flowers borne in terminal cones. Deep red and green-coloured leaves surround the female flower in 'Silvan Red', and extend down the stem forming an attractive display (Fig. 1).

Preliminary storage trials indicated that vase life of 'Silvan Red' was significantly reduced after 42 days dry storage at 1°C, primarily due to an increase in leaf desiccation (Jones and Faragher, 1991). Leaf desiccation during storage in *Leucadendron* 'Silvan Red' was significantly inhibited by a pre-storage pulse with 20% sucrose and 100 mg l^{-1} sodium dichloroisocyanurate (DICA) (Jones, 1991). This paper further characterises the beneficial effects of pre-storage sucrose pulses on cut *Leucadendron* foliage during dry storage.

2. Materials and methods

Plant material

Stems of *Leucadendron* 'Silvan Red' were harvested and transported to the laboratory in water within 1 h. Vase life was considered terminated by pronounced leaf desiccation, which appeared as small areas of dry, necrotic tissue, in ≥5 leaves. Those stems showing signs of leaf desiccation immediately after dry storage were discarded, and vase life, therefore, was only assessed on unmarked stems. Each treatment was replicated at least ten times and each experiment was repeated at least twice.

Storage trials

Stems were trimmed (in air) to 30 cm, weighed and placed in individual test-tubes containing solutions of: 100 mg l^{-1} DICA as a germicide, and 2, 5, 10, 20, 30 or 40% sucrose. Stems were pulsed for 24 h at 1 or 20°C; solutions were precooled to 1 or 20°C (representing cool store and room temperatures, respectively) before stems were pulsed. Fresh weight of individual stems was measured by weighing stems and the solution (+ test-tube) before and after the pre-storage pulse.

Stems were thoroughly sprayed with iprodione (wettable powder, 50% a.i.; commercial name: Rovral, Rhone Poulenc, Australia) to guard against fungal attack during storage, then wrapped in newsprint and 35 μm polyethylene plastic and placed in a dark coolroom set at 1°C and 90% relative humidity (RH) for 42 days. After storage, stems were assessed for leaf desiccation, weighed, and 20 mm was cut from the base of each stem before being placed in distilled water at 1°C for 24 h as a rehydration treatment. Stems were then placed in distilled water under constant conditions (20°C, 65% RH, under constant cool fluorescent light of 10 μmol m^{-2} s^{-1}) for vase-life assessment. Fresh weight and vase life were assessed daily by weighing each flower stem, and inspecting leaves for signs of desiccation.

The possible osmotic effects of sucrose pulses were tested by comparing the effects of sucrose with sorbitol and mannitol. Stems were placed in 0, 10, 20, 30 or 40% sucrose, sorbitol or mannitol (BDH, Poole) for 24 h at 1°C, stored and assessed as described above.

Total soluble sugar analysis

Total sugars were assayed before and after storage in stems pulsed with 0, 10, 20, 30 and 40% sucrose for 24 h at 1°C. Tissue was freeze-dried and total soluble sugars were analysed after Haslemore and Roughan (1976). Sugar content was also assayed in stems pulsed with 20% sucrose at 1 and 20°C for 0, 2, 4, 6, 8, 16, 20 and 24 h. Replicate (pulsed) stems were stored for 42 days at 1°C and assessed for leaf desiccation after storage.

Distribution of ^{14}C-sucrose during pulse treatment

Stems of 'Silvan Red' were trimmed to 30 cm and placed in pulse solutions of distilled water or 20% sucrose containing 1 μCi ml^{-1} [U-^{14}C] sucrose (Amersham, Sydney, Australia) within 1 h of harvest. The amount of labelled sucrose added to

distilled water (30 μl) was low enough to be considered negligible, and the sucrose concentration of the distilled water solution was therefore considered to be 0%. Stems were pulsed for 24 h and discs (1 g) were cut from leaf and flowerhead tissue and placed in 10 ml of scintillation liquid (ReadySafe, Beckman, Fullerton, USA). Radioactivity was measured on a LKB Wallac 1215 Rackbeta scintillation counter (LKB Wallac, Finland).

3. Results

Storage trials

Approximately 30% of untreated *Leucadendron* 'Silvan Red' stems displayed severely desiccated leaves after 42 days dry storage at 1°C (Fig. 2). A pre-storage sucrose pulse of 20% at 1°C resulted in the complete inhibition of leaf desiccation (Fig. 2). The temperature at which the pre-storage sucrose pulse was conducted was significant as sucrose pulses of ≥10% applied at 20°C resulted in a sharp increase in leaf desiccation (Fig. 2).

Post-storage vase life, assessed by determining leaf desiccation each day after storage, was slightly improved in stems pulsed with 20 or 30% sucrose for 24 h at 1°C compared with stems pulsed with distilled water (Fig. 3). Lower concentrations of sucrose (2 and 5%; Fig. 3), as well as higher pulse concentrations (40%) had little effect on post-storage vase life. In an effort to determine the possible osmotic effects of sucrose pulses, stems were pulsed with 10–40% sorbitol or mannitol (Fig. 3). Leaf desiccation was not observed immediately after storage in stems treated with

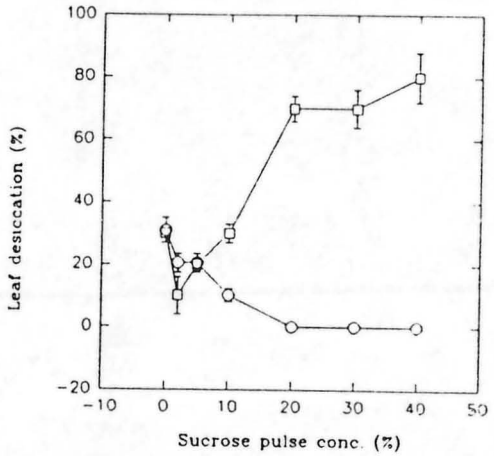

Fig. 2. Effect of a 24 h pre-storage sucrose pulse (0, 2, 5, 10, 20, 30 and 40%) at 1°C (○) or 20°C (□) on leaf desiccation in *Leucadendron* 'Silvan Red' during 42 days storage at 1°C. Leaf desiccation is expressed as a percentage of total number of leaves ($n = 20$). Bars represent SE.

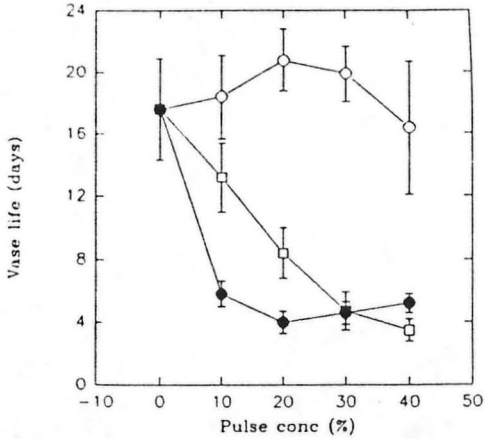

Fig. 3. Effect of a pre-storage 24 h pulse at 1°C of sucrose (○), sorbitol (□) and mannitol (●) (at 0, 10, 20, 30, and 40%) on vase life, determined by leaf desiccation, of *Leucadendron* 'Silvan Red' after 42 days storage at 1°C. Bars represent SE.

sorbitol or mannitol (data not shown), but appeared rapidly after storage, resulting in a significant decline in vase life (Fig. 3).

Total soluble sugar analysis

Total soluble sugar content of 'Silvan Red' leaves declined significantly during 42 days dry storage at 1°C (Table 1). Loss of total sugar content was markedly higher in untreated leaves or in leaves pulsed with 10% sucrose before storage. The decline in sugar content was significantly reduced in leaves treated with ≥20% sucrose pulses.

Table 1
Depletion of total soluble sugar content in leaves of *Leucadendron* 'Silvan Red' during 42 days storage at 1°C. Stems were pulsed before storage in 0, 10, 20, 30, and 40% sucrose for 24 h at 1°C. Total soluble sugar content (after Haslemore and Roughan, 1976) was determined after pulsing, and after 42 days dry storage at 1°C. Depletion of sugar is expressed in mg g^{-1} dry weight, and as a percentage of loss (based on sugar content after pulsing). Each value represents the mean of 20 leaves, and the experiment was repeated twice.

Pulse concentration (%)	Total sugar loss (mg g^{-1})	Total sugar loss (%)
0	18.2 a	34.0 a
10	20.4 a	35.9 a
20	7.2 b	13.3 b
30	7.2 b	12.9 b
40	5.2 b	9.0 b

Values in the same column with differing letters are significantly different at $P = 0.05$.

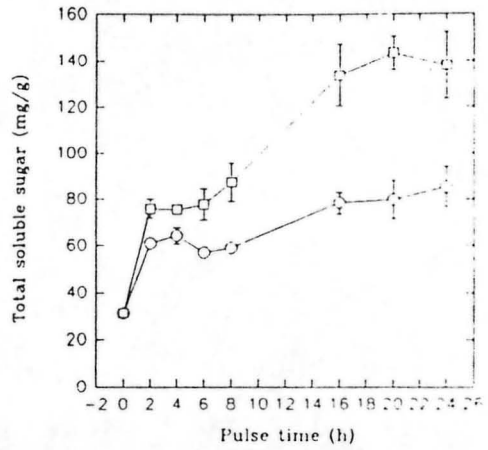

Fig. 4. Total soluble sugar levels (mg g^{-1}) in *Leucadendron* 'Silvan Red' leaves during a 24 h pulse in 20% sucrose at 1°C (○) and 20°C (□). Stems were removed from the pulse solution after 2, 4, 6, 8, 16, 20 and 24 h. Leaves were stripped from stems, freeze-dried and assayed for total soluble sugar content after Haslemore and Roughan (1976) ($n = 20$). Bars represent SE.

Sugar levels declined only marginally (5.2 mg g^{-1}, or 9%) in leaves treated with a 40% pre-storage pulse, for example, compared to 18.2 mg g^{-1} (or 34%) in untreated leaves (Table 1).

A time course of total soluble sugar content in leaf tissue pulsed with 20% sucrose at 1 and 20°C is shown in Fig. 4. Uptake of the sucrose solution resulted in a rapid increase in total sugar content in the first two hours of the pulse treatment: 29.6 mg g^{-1} in leaves pulsed at 1°C, and 44.4 mg g^{-1} in leaves pulsed at 20°C (Fig. 4). This amounts to 54.8 and 42.3%, respectively, of the total sugar uptake over 24 h. Total sugar content increased to a level more than 60% higher in leaves pulsed at 20°C than at 1°C.

Distribution of ^{14}C-sucrose after pulse treatment

The distribution of the pulse solution within leaves and flower tissues of 'Silvan Red' during a 24 h pulse at 20°C was investigated using ^{14}C-sucrose (Table 2). Over 3 times more radioactivity was recorded in the flower heads than in the leaves of 'Silvan Red' stems pulsed with labelled distilled water, in contrast to stems pulsed with labelled 20% sucrose, where 2.3 times more radioactivity was recorded in the leaves than in the flowerheads (Table 2). Making due allowance for the balance between the weights of flowerheads and leaves, label uptake by stems pulsed with 20% sucrose was significantly higher (167% increase) than when pulsed with label alone.

4. Discussion

Uptake trials with ^{14}C-sucrose (Table 2) indicated a fundamental change in the pattern of pulse solution distribution when sucrose was applied. Labelled distilled water was directed predominately to the flowerhead, while labelled 20% sucrose was distributed primarily to the leaves. A similar pattern was reported in cut rose flowers during a 1 h pulse with ^{14}C-sucrose (2%), which was directed exclusively to the leaves and stem tissue of cut roses (Sacalis and Durkin, 1972). The labelled sucrose was later redistributed to the flowerhead when the rose flowers were placed in distilled water. This change in vase solution distribution indicates that a significant percentage of exogenously applied sucrose reached the leaf tissue and was available to protect against desiccation during dry storage.

Sucrose concentration did not affect stem fresh weight during the 24 h pulse, nor did it affect solution uptake in a 24 h rehydration phase after storage (data not shown). Significantly, pulsing stems with 10–40% sorbitol or mannitol also had no effect on fresh weight or solution uptake before or after storage (data not shown), but adversely affected vase life, due to an increased and accelerated incidence of leaf desiccation (Fig. 3). These results indicate that the beneficial effects of sucrose on stored 'Silvan Red' stems were not due to improved leaf hydration prior to dry storage.

Pulsing with sucrose before storage appeared to inhibit the rate of carbohydrate depletion in 'Silvan Red' leaves during storage (Table 1). Prolonged cool storage generally results in accelerated senescence and membrane degradation in cut flower tissues (Goszczynska and Rudnicki, 1988), primarily due to the depletion of carbohydrate reserves. Glucose and fructose levels, for example, declined significantly in anthurium flowers stored at 4°C (Pritchard et al., 1991). Total sugar levels declined in a similar manner in 'Silvan Red' leaves during 42 days dry storage (Table 1). Although the storage temperature (1°C) was low, metabolic activity within the leaves would probably continue at a reduced rate, resulting in the reduction of carbohydrates.

The decline in sugar levels was significantly less in leaves treated with ≥20%

Table 2
Levels of ^{14}C expressed as absolute values (counts per minute/g fresh weight) and percentage of total activity detected (in brackets) in the leaves and flowerheads of *Leucadendron* 'Silvan Red' after a 24 h pulse at 20°C in 0% (distilled water + 1 μCi ml^{-1} ^{14}C-sucrose) or 20% sucrose + 1 μCi ml^{-1} ^{14}C-sucrose. Mean fresh weight of the leaves and flowerheads used in both treatments is followed by SE (in brackets).

Sucrose concentration (%)	Leaf	Flowerhead
0	194.9 a	619.4 b
	(10.1%)	(32.2%)
20	770.4 b	338.9 a
	(40.0%)	(17.7%)
Fresh weight	6.17 (0.74)	3.94 (0.51)

Values in the same column with differing letters are significantly different at $P = 0.05$.

sucrose before storage than in untreated leaves (13% c.f. 34%; Table 1). Although treated leaves contained more sugar before storage, they lost proportionally less. It is possible that exogenous sucrose interacted directly with membranes, as suggested by Goszczynska et al. (1990), and was not available for metabolic activity. A direct interaction of sucrose with cellular membranes could also explain the inhibitory effect of sucrose on leaf desiccation.

The exact manner in which applied sucrose protected 'Silvan Red' leaves from desiccation during long-term dry storage (Fig. 2) is not known and requires further investigation. A 24 h pulse of 20% sucrose protected carnation petals against freezing injury during five days at −4°C (Heins et al., 1981). The lowest temperature recorded during the storage period in our trials was −0.4°C (data not shown), not low enough to cause transient freezing and damage to leaf tissue. Soluble sugars are also known to protect membranes during seed desiccation or freezing by preventing membrane fusion, phase transition and phase separation (Crowe and Crowe, 1986; Caffrey et al., 1988; Crowe et al., 1988; Crowe and Crowe, 1992). Similarly, applied sucrose effectively stabilised spinach membranes during low temperature stress (Santarius, 1973) and, at 5–10%, protected lipid vesicles from dehydration (Strauss and Hauser, 1986).

In cut flowers, applied sucrose has many functions, such as aiding in flower opening (Evans and Reid, 1988; Van Doorn et al., 1991) and maintaining the membrane integrity of petal tissue (Halevy and Mayak, 1979). The water-holding capacity of carnation petals was, concomitantly, dependent on membrane integrity (Borochov and Woodson, 1989). Exogenous sucrose also reduced the age-induced increase in membrane lipid microviscosity in rose petals (Goszczynska et al., 1990). Our results suggest the possibility that applied sucrose acted in a similar manner in *Leucadendron* leaf tissue during dry storage.

This treatment could be applicable to other *Leucadendron* species with a shorter vase life, notably *L. salignum*, *L. laureolum*, *L. orientale*, and *L. discolor*. Sucrose pulsing would be particularly useful in ensuring successful storage and transport in these species, and in extending the marketing period of *Leucadendron* foliages with a shorter flowering period.

Acknowledgements

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References

Acock, B. and Nichols, R., 1979. Effects of sucrose on water relations of cut, senescing carnation flowers. Ann. Bot., 44: 221–230.

Borochov, A. and Woodson, W.R., 1989. Physiology and biochemistry of flower petal senescence. Hortic. Rev., 11: 15–43.

Caffrey, M., Fonseca, V. and Leopold, A.C., 1988. Lipid–sugar interactions. Plant Physiol., 86: 754–758.

Crowe, J.H. and Crowe, L.M., 1986. Stabilization of membranes in anhydrobiotic organisms. In: A.C. Leopold (Editor), Membranes, Metabolism, and Dry Organisms. Cornell University Press, Ithaca, N.Y., pp. 188–209.

Crowe, J.H. and Crowe, L.M., 1992. Membrane integrity in anhydrobiotic organisms: toward a mechanism for stabilizing dry cells. In: G.N. Somero, C.B. Osmond and C.L. Bolis (Editors), Water and Life. Springer-Verlag, Berlin, pp. 87–103.

Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J. and Anchordoguy, T.J., 1988. Interactions of sugars with membranes. Arch. Biochem. Biophys., 947: 367–384.

Evans, R.Y. and Reid, M.S., 1988. Changes in carbohydrates and osmotic potential during rhythmic expansion of rose petals. J. Am. Soc. Hortic. Sci., 113: 884–888.

Goszczynska, D.M. and Rudnicki, R.M., 1988. Storage of cut flowers. Hortic. Rev., 10: 35–62.

Goszczynska, D.M., Itzhaki, H., Borochov, A. and Halevy, A.H., 1990. Effects of sugar on physical and compositional properties of rose petal membranes. Sci. Hortic., 43: 313–320.

Halevy, A.H., 1976. Treatment to improve the water balance of cut flowers. Acta Hortic., 64: 223–230.

Halevy, A.H. and Mayak, S., 1979. Senescence and postharvest physiology of cut flowers, Part 1. Hortic. Rev., 1: 204–236.

Halevy, A.H. and Mayak, S., 1981. Senescence and postharvest physiology of cut flowers, Part 2. Hortic. Rev., 3: 59–143.

Haslemore, R.M. and Roughan, P.G., 1976. Rapid chemical analysis of some plant constituents. J. Sci. Food Agric., 27: 1171–1178.

Heins, R.D., Howell, G.S. and Wilkins, H.F., 1981. The influence of sucrose, ethanol and calcium nitrate on the freezing point and long-term low-temperature storage of carnation flowers. Sci. Hortic., 14: 269–275.

Jones, R.B., 1991. A pre-storage sucrose pulse protects cut *Leucadendron* var. 'Silvan Red' during long-term dry storage at 1°C. Acta Hortic., 298: 247–254.

Jones, R.B. and Faragher, J.D., 1991. Cold storage of selected members of the Proteaceae and Australian native cut flowers. HortScience, 26: 1395–1397.

Pritchard, M.K., Hew, C.S. and Wang, H., 1991. Low-temperature storage effects on sugar content, respiration and quality of anthurium flowers. J. Hortic. Sci., 66: 209–214.

Sacalis, J.N. and Durkin, D., 1972. Movement of ^{14}C in cut roses and carnations after uptake of ^{14}C-sucrose. J. Am. Soc. Hortic. Sci., 97: 481–484.

Santarius, K.A., 1973. The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. Planta, 113: 105–114.

Strauss, G. and Hauser, H., 1986. Stabilization of small unilamellar phospholipid vesicles by sucrose during freezing and dehydration. In: A.C. Leopold (Editor), Membranes, Metabolism, and Dry Organisms. Cornell University Press, Ithaca, N.Y., pp. 318–326.

Van Doorn, W.G., Groenewegen, G., Van de Pol, P.A. and Berkholst, C.E.M., 1991. Effects of carbohydrate and water status on flower opening of cut Madelon roses. Postharvest Biol. Technol., 1: 47–57.

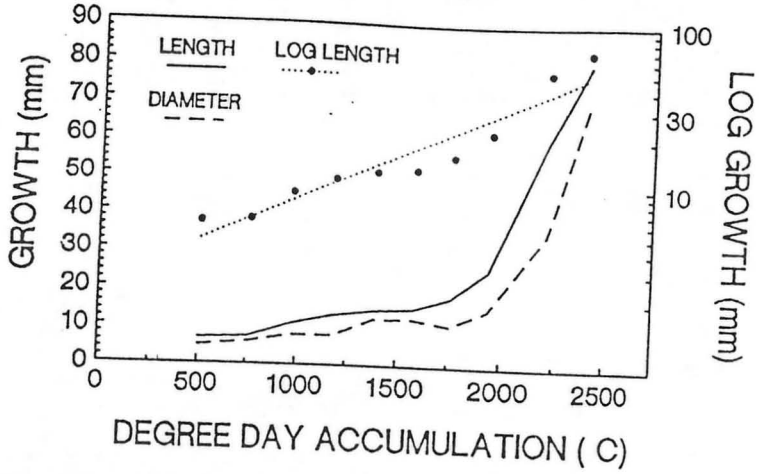

Figure 6. Dimensions of the flower bud plotted against degree day accumulation (^{o}C) over a base of 6^{o}C. September 1 represents the zero point at which accumulation began while 2400 units were accumulated by February 15. The regression plot of bud length on degree day accumulation is is nearly linear.

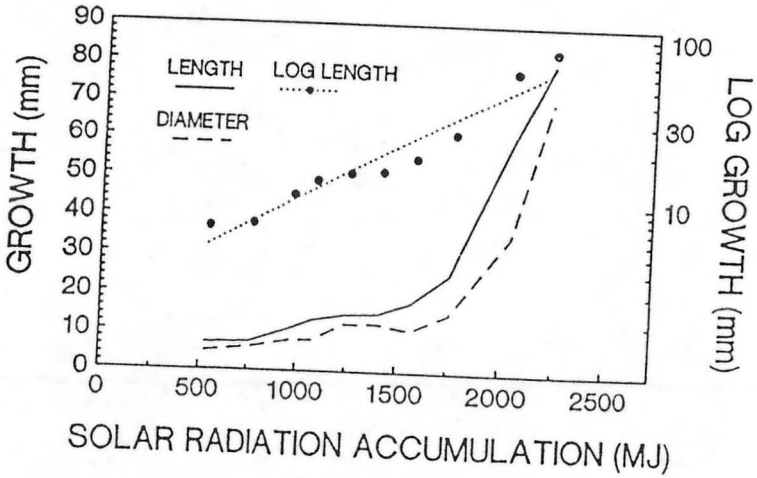

Figure 7. Dimensions of the flower bud plotted against daily solar radiation accumulation (MJ). September 1 represents the zero point at which accumulation begain while 2225 MJ were accumulated by February 15. The regression plot of bud length on solar radiation accumulation is nearly linear.

FLOWER INITIATION IN PROTEA AND TELOPEA

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Abstract

Growth measurements of Protea neriifolia 'Salmon Pink' and Protea cynaroides 'Long Leaf' after flowering indicate multiple flushes of growth. Floral initiation occurs in P. neriifolia after the first spring flush. P. cynaroides may initiate flower heads only during two short periods after the two flushes each year. Floral primordia of Telopea speciosissima were examined using dissection and scanning electron microscope techniques.

1. Introduction

Protea neriifolia and Protea cynaroides seedlings flower only after passing through a juvenile phase. As seedlings grow through the juvenile phase, branching occurs and stems thichen, given good growing conditions. Root growth must occur rapidly to support both the shoot and future inflorescence development. A seedling of Protea neriifolia will produce its first flowers usually two or three years from planting. The Long Leaf variety of Protea cynaroides often produces one inflorescence three years from planting, although flowering occasionally occurs in two years. Plants of P. neriifolia grown from cuttings may flower as rooted cuttings if mature wood is present.

The Protea cut flower industry must depend on a sufficient supply of flowers with good quality blooms. The current market supply for many Proteaceous species varies considerably during the year with shortages of flowers at some periods and a glut at others. As part of understanding the control of flowering it is important to establish when flower initiation occurs in the annual cycle for each species.

The current work investigates the initiation of flowering in Protea neriifolia 'Salmon Pink', Protea cynaroides 'Long Leaf' and Telopea speciosissima. This has been done by dissection studies and electron microscopy.

2. Materials and Methods

Both vegetative and reproductive shoot tips of Protea neriifolia 'Salmon Pink' and the Long Leaf varieant of Protea cynaroides have been dissected. The 'Salmon Pink' selection has inflorescences which grow above the terminal leaves, and medium sized flowers. The color of the outer bracts is a soft pastel pink. The flowers show very little bract burning or curling. The clone originated in Victoria, where it flowers from April to November.

Shoot and flower tips were collected from 2 year old Protea neriifolia 'Salmon Pink' and Protea cynaroides and 4 year old Telopea speciosissima grown in the field at a plantation near Peats Ridge, New South Wales. After the autumn flowering season in 1988, five shoots on each of 20 Protea neriifolia 'Salmon Pink' and Protea cynaroides were labelled in May. During the middle of each month vegetative growth on each branch was recorded. Inflorescence bud development was noted on all plants. Floral buds collected from T. speciosissima shoots were dissected and all bracts removed. Resin moulds of the shoot tips were made by impressions using Spurr's resin. The floral apices were coated with platinum or gold and then examined under the scanning electron microscope.

3. Results

Both Protea showed a spring growth flush, with other flushes during the year (Figs. 1, 2). P. neriifolia 'Salmon Pink' grew with successive flushes during the summer, while P. cynaroides showed a significant reduction in growth during December (summer). Flower buds of P. neriifolia 'Salmon Pink' were found to be initiated from late October to early November at Peats Ridge. A receptacle begins to develop on the end of terminal shoots (Figs. 3, 4) which then develops into determinate flowers. Early during initiation a few flower buds abort with no flower. These shoots were observed not to develop further. By early December the flower buds had completed initiation and "bypass" or lateral shoots began to develop. In February some early flowers had black fur tips on the outer bracts.

Mature plants of P. cynaroides 'Long Leaf' developed flower buds during May with another initiation period in December. During both these times some bracts appeared to be remnants of floral buds which grew into vegetative shoots. A receptacle develops under the apical meristem as the stem widens. Bract development, flower head enlargement and elongation occurred over a 5-6 month period.

Shoot tips collected from Telopea speciosissima showed the first evidence of initiation in mid December (Figs. 5, 6). Floral buds developed more rapidly on older shoots as compared to the current season's growth. Floral primordia emerged from mid January to February. Pairs of florets differentiated as a new floral cone developed (Figs. 7, 8).

4. Discussion

Protea neriifolia 'Salmon Pink' has a definite cycle of growth and flowering. Flower initiation occurs just after the first main flush of growth in spring. Two further flushes of side shoots or small stems will occur given adequate moisture. The abortion of flower heads occurs only within a limited time, perhaps due to higher than normal temperatures or moisture relations at that time. During winter, shoots grow very little and flowering of the previous year's buds continues at a slow rate.

The initiation of flower heads in Protea cynaroides 'Long Leaf' seems to occur for short periods twice a year. The apical meristem has the ability to set quickly or to revert to vegetative growth. Further research is warranted to determine how and why this occurs. It is not uncommon to find inflorescences with leaves which appear as bracts.

Telopea speciosissima has a primary flush of growth from November to January. Floral primordia initiate over a 6-8 week period after which there may be another vegetative flush of growth on the plants. A few plants flower in March and April which may mean that flowering may be changed given a certain stage of growth or conditions.

Acknowledgements

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References

Jacobs, G., 1983. Flower initiation and development in Leucospermum cv. Red Sunset J. Amer. Soc. Hort. Sci. 108:32-35.

Vogts, M. 1982. 'Proteaceae: Know Them and Grow Them'. Proteaflora Enterprises Pty. Ltd. Melbourne p. 91-105, 224-227.

Zieslin, N. and Moe, R., 1985. Rosa In 'Handbook of Flowering' (ed. Halevy, A.H.) CRC Press, Inc. Boca Raton, Florida. p. 214-225.

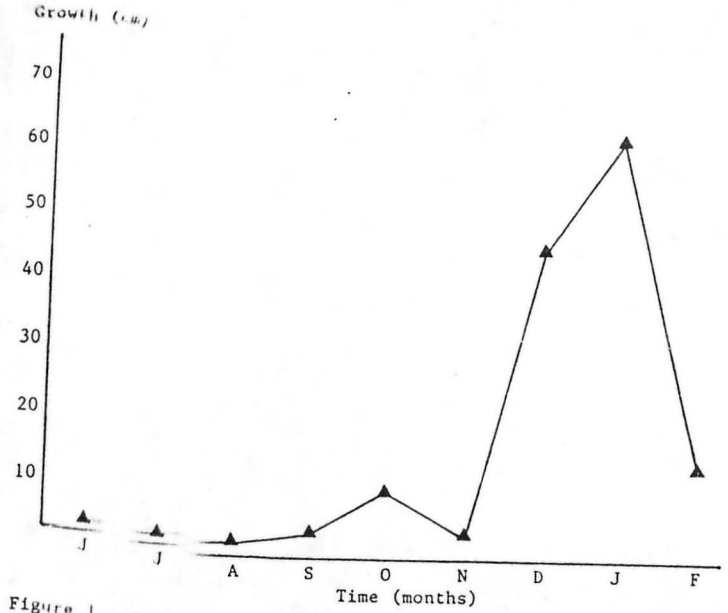

Figure 1 Total amount of vegetative growth of Protea neriifolia 'Salmon Pink' on 20 two year old clonal plants.

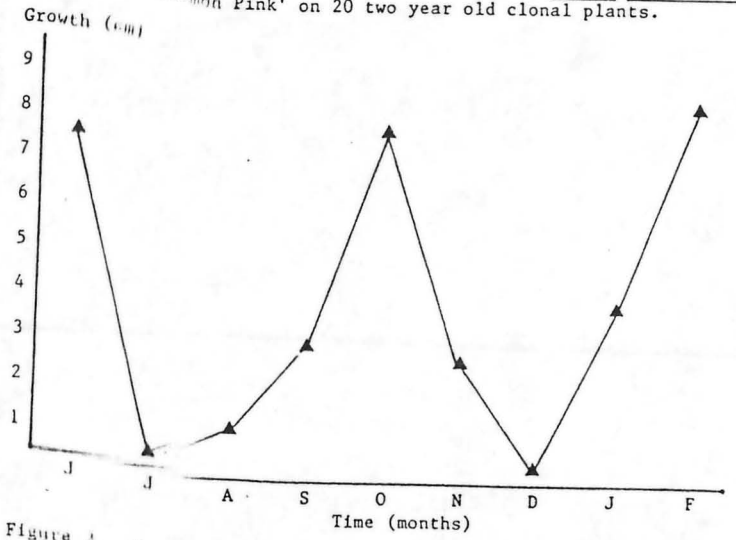

Figure 2 Total amount of vegetative growth of Protea cynaroides 'Long Leaf' on 20 two year old seedlings at Peats Ridge.

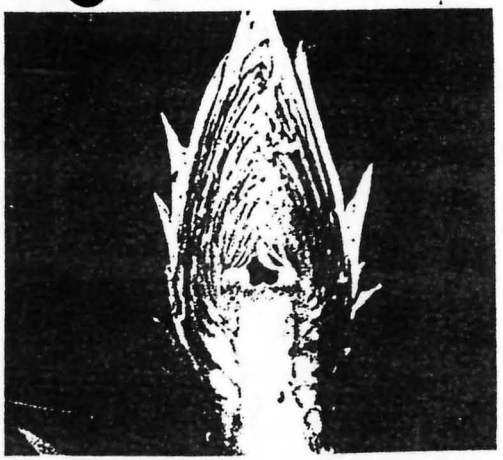

Figure 3 - Flower head of Protea cynaroides 'Long Leaf' variant with developing flowers on receptacle.

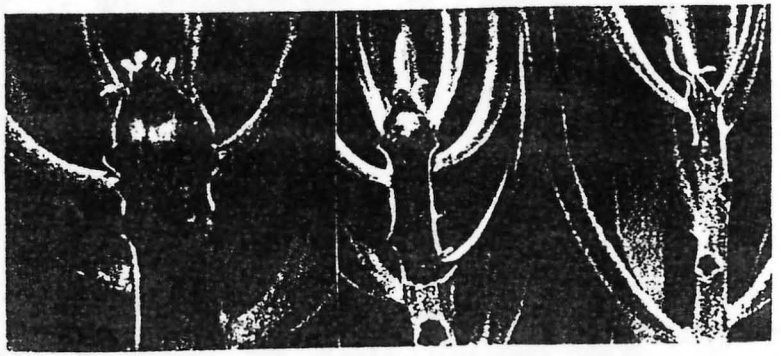

Figure 4 - Close up (left) and comparison of Protea neriifolia 'Salmon Pink' floral and vegetative shoots in early November at Peats Ridge, New South Wales.

Figure 5 - Scanning electron micrograph of the apical meristem of floral primordia of Telopea speciosissima.

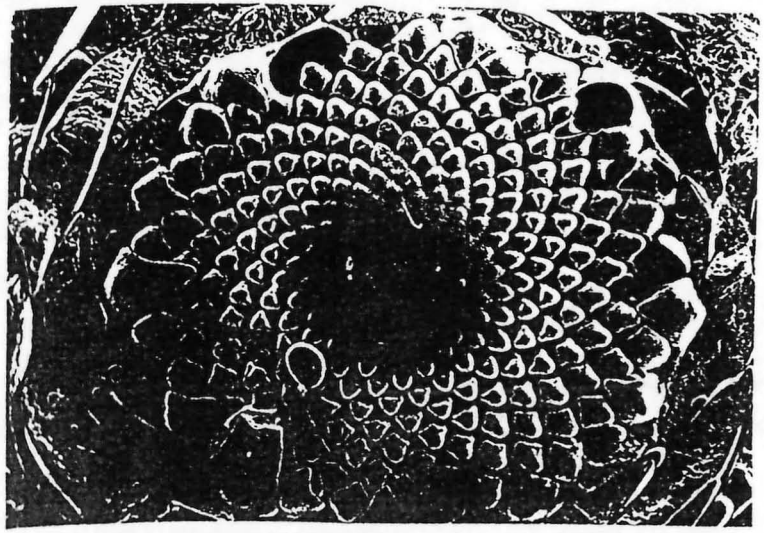

Figure 6 - Scanning electron micrograph of differentiating bracts and florets of T. speciosissima.

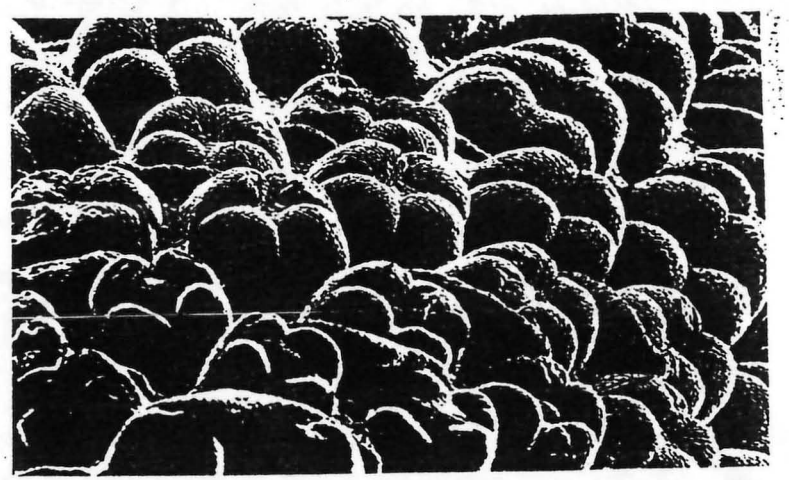

Figure 7 - Scanning electron micrograph of individual flowers developing in February on T. speciosissima apical meristem.

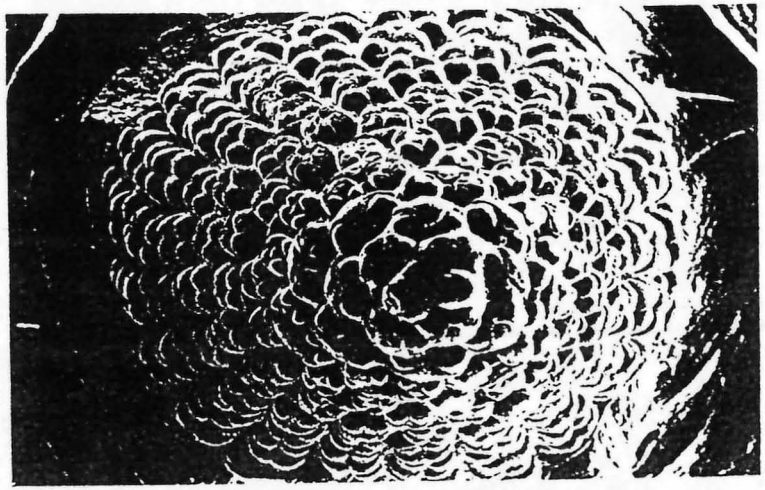

Figure 8 - Scanning electron micrograph of T. speciosissima floral head showing developing paired flowers.

EFFECT OF TEMPERATURE, DAYLENGTH AND GROWTH REGULATORS ON FLOWERING IN PROTEA, TELOPEA AND LEUCOSPERMUM

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Abstract

Flowering of 27 clonal plants of Protea neriifolia 'Salmon Pink' was recorded at each of four Protea plantations in southeastern Australia. Significant differences have been found in both period of flowering and numbers of blooms. Flowering of Telopea speciosissima was recorded on tagged plants at five field sites. A warm winter advanced and enhanced flowering more so in cooler areas. Treatments with gibberellic acid and cytokinins applied in July promoted early flowering in Leucospermum cordifolium. Disbudding delayed flowering by two to three weeks.

1. Introduction

Plants grow and flower at different periods of time in response to the environment in field produced Protea. The research described in this paper aims to establish a flowering date base for four locations and to determine what environmental factors determine the time of flowering in Protea neriifolia 'Salmon Pink', Telopea speciosissima and the Long Leaf variant of Protea cynaroides.

A separate experiment has been conducted to test the effects of disbudding and growth regulators on the flowering of Leucospermum cordifolium. Other researchers have found that flowering in other Leucospermum can be delayed by deheading (Jacobs and Honeyborne, 1978 and Brits, 1986) or by using ethephon and that growth regulators can alter flowering (Jacobs, Napier and Malan, 1986 and Halevy, 1983). The flowering of secondary inflorescences of Leucospermum was found to depend on the mean daily temperature (Jacobs and Honeyborne, 1979).

2. Materials and Methods

The experiments were conducted at five separate commercial Protea plantations in coastal New South Wales and southeastern Queensland. The geographical and soil data are given in Table 1. All the areas receive summer rainfall with spring being the driest period of the year. The maximum and minimum daily temperatures and precipitation recorded at each site.

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Protea Research

2.1 Protea Phenology

During April, 1987, 27 one year old clonal plants of Protea neriifolia 'Salmon Pink' were planted at each plantation except site No. 2 in a complete random design. All plants were watered after planting and thereafter received only natural precipitation. Weedmat or mulch is used to control weeds. One year after planting, selective pruning of weak or low branches was carried out so that each plant had 12 to 22 branches at least 20 cm long. The flowering date of each inflorescence was recorded as the time when the inner bracts of each flower head began to separate at the top of the inflorescence. After flowering each inflorescence was pruned, removing as little of the stem as possible.

Table 1 - Location and Soil Type of Five Protea Plantations in Southeastern Australia

Characteristic	Plantation No. 1 Springbrook Queensland	No. 2 Taree, N.S.W.	No. 3 Peats Ridge N.S.W.	No. 4 Kurrajong N.S.W.	No. 5 Robertson N.S.W.
Latitude	28°14'S	31°52'S	33°19'S	33°30'S	34°35'S
Longitude	153°17'E	152°28'E	151°15'E	150°35'E	150°37'E
Altitude (m)	610	170	280	580	740
Aspect	southeast	north	northeast	east	east
Soil Type	reddish silty clay loam	brown sandy clay loam	yellow sandy loam	dark silty clay loam	reddish sandy clay loam
Soil pH	5.0	4.7	5.2	4.8	4.8

2.2 Telopea Phenology

Healthy 3-5 year old seedling Telopea speciosissima were chosen in April, 1987 to record flowring using metal labels. Plants were selected at random at all five plantations and observations of flowering were made during spring 1988. Each inflorescence was considered to be flowering when the first style was released on the flower head. Some dates were estimated due to time and distance factors. The inflorescences were picked by growers and stems cut back severely on each mother plant.

2.3 Growth regulator treatments

Ten flower buds on each of four plants of Leucospermum cordifolium were sprayed with a number of growth regulator treatments on 25 July 1988 between 10am and 1pm. All plants were at plantation No. 4 near Kurrajong Heights, New South Wales. Shoots with single dominant flower buds were treated at random on each plant.

The treatments consisted of: 1) a free flowering control 2) 250 mg/l of Grocel GA_3 (active ingredient: 100g/kg Gibberellic acid 3) 1.25 ml/l of Cytokin (GA_{4+7}) 4) 0.025 g/l of benzyl aminopurine (BA) 5) disbud the terminal bud of the shoot 6) 125ml/l of Bonzi (active ingredient: 4g/l Paclobutrazol) 7) 200 ml/100l of Pro-Gibb (active ingredient: 100g/l Gibberellic acid and 8) disbud the terminal bud and spray with Cytokin (1.25 ml/l). Dates of flowering were recorded as the first style was released on each inflorescence.

3. Results

3.1 Protea Phenology

The flowering of Protea neriifolia 'Salmon Pink' is summarized in Figure 1. The first flower opened at Peats Ridge in March, 1988. The last flower to bloom was at Robertson during mid October.

Plants at Springbrook produced the most flowers as shown in Table 2. The average flowers per plant at Springbrook was 4.3 compared to 1.0 flower per plant at Robertson.

3.2 Telopea Phenology

Bloom periods for the Telopea speciosissima plants are given in Figure 2. First flowers occurred in Springbrook where plants had the greatest number of blooms per plant. Table 3 indicates that an uneven number of plants were observed at each site.

3.3 Growth Regulator Treatments

The flowering of each plant of Leucospermum cordifolium is shown in Table 4. The control flowered under natural conditions. In contrast, shoots with Grocel, Pro-Gibb and Bonzi partly became vegetative during November or ceased development. Disbudding the primary bud resulted in the most consistent delay in flowering.

4. Discussion

The flowering of Protea neriifolia 'Salmon Pink' shows highly significant differences in flowering between four commercial plantations in terms of number of blooms and the period of flowering. This clone generally flowers in autumn but due to cool winter temperatures flower rate of development slows considerably until September. It may be possible to alter flowering by either pruning or chemical means.

Further flowering data is required for Telopea speciosissima to determine the effects of climate from one year to another. The species produces many flowers within a very short period. This will cause more difficulty in attempts to extend the flowering period. However, some plants flower in autumn. The 1988 season was wet which increased bud rot in both plantations 1 and 2. The warm winter advanced flowering more in the southern districts. Inflorescence development should not be related to daylength whereas flower initiation may be.

Leucospermum cordifolium flower buds do respond to some growth regulators by promoting or delaying flowering. Both cytokinins and ProGibb applied to flower buds advanced flowering by one to three weeks. Disbudding may be used to delay flowering by two to three weeks but is less effective as the spring temperatures increase.

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References

Brits, G. J. 1986. Extension of harvesting period in Leucospermum by means of manual and chemical pruning methods. Acta Hortic. 185:237-240.

Halevy, A. H. 1983. Regulation of flowering in flower crops by growth substances. Acta Hortic. 147:193-197.

Jacobs, G. 1985. Leucospermum. IN Handbook of Flowering (Ed. Halevy, A. H.) CRC Press Inc., Boca Raton, Florida. pp. 283-286.

Jacobs, G. and Honeyborne, G. E. 1978. Delaying the flowering time of Leucospermum cv. Golden Star by deheading. Agroplantae 10:13-15.

Jacobs, G. and Honeyborne, G. E. 1979. The relationship between heat unit accumulation and the flowering date of Leucospermum cv. Golden Star. Agroplantae 11:83-85.

Jacobs, G. Napier, D. N. and Malan, D. G. 1986. Propects of delaying flowering time of Leucospermum. Acta Hortic. 185:61-65.

Napier, D. N., Malan, D. G., Jacobs, G. and Bernitz, J. W. 1986. Improving stem length and flower quality of Leucospermum with growth regulators. Acta Hortic. 185:67-73.

Nixon, P. 1987. The Waratah. Kangaroo Press. Kenthurst, NSW. pp. 11-30.

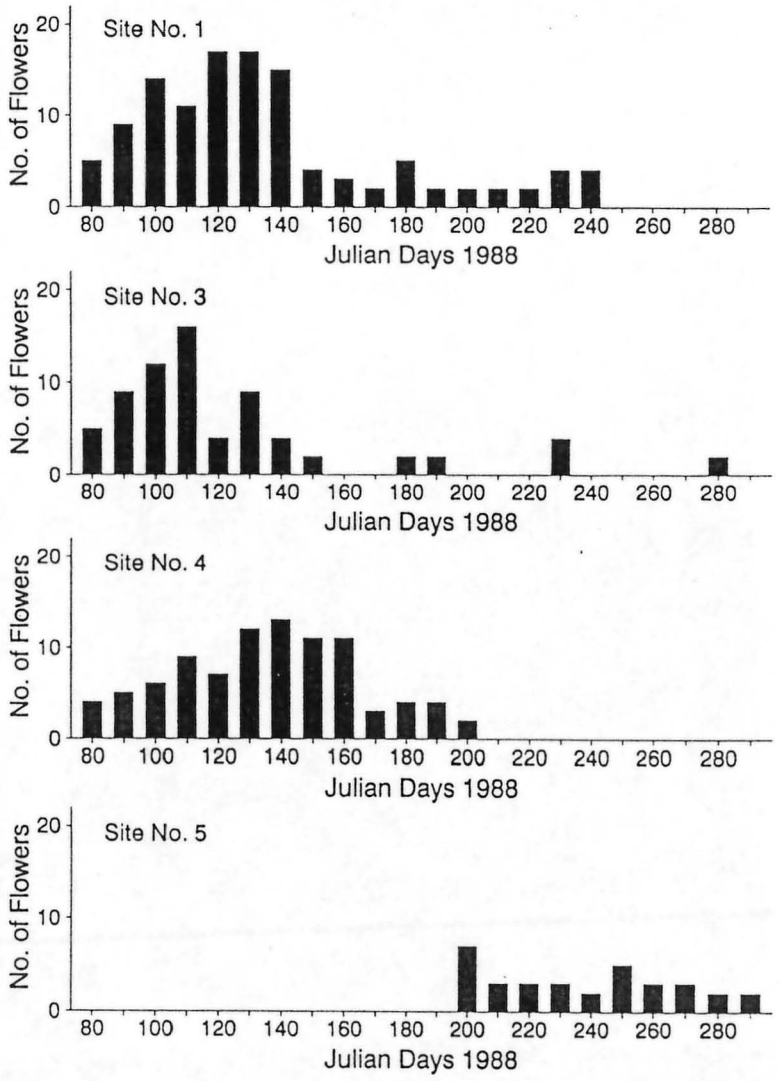

Figure 1. The flowering of Protea neriifolia 'Salmon Pink' at four protea plantations during 1988.

Table 2 - Data Analysis of Protea neriifolia 'Salmon Pink' flowering for 1988.

Site	Mean in Julian D.	S.E. mean	Number of Flowers	Flower size(mm)	Abortions
1	124	3.21	112	124	2
3	113	4.30	68	116	3
4	132	2.78	90	121	2
5	232	5.81	26	108	5
Total			296		12

Table 3 - Comparison of Flowering of Telopea speciosissima at five sites in southeastern Australia

Site	Mean in Julian D.	S.E mean	Number of Plants	Number of Flowers	F Value
1	250	6.77	25	249	15.45
2	268	9.30	24	133	16.84
3	257	5.25	57	249	5.12
4	266	4.89	48	249	14.69
5	268	4.88	57	268	6.97

Grand Flowering Mean: 263

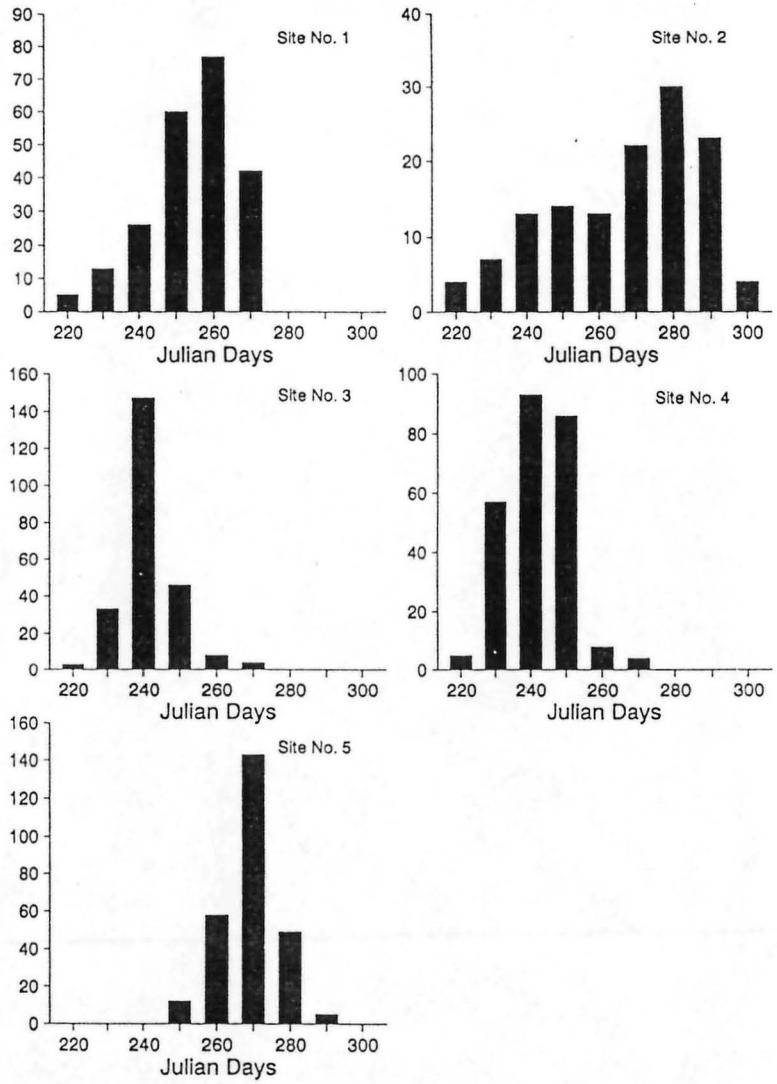

Figure 2. The rate of flowering of Telopea speciosissima at five protea plantations during 1988.

Table 4 - The effect of growth regulators on flowering of Leucospermum cordifolium.

Treatment	Days of Flower of all Plants (Plant Number) 1	2	3	4	S.E.
1	292	304	298	291	2.95
2	280	298	300	293	3.77
3	285	290	285	288	3.70
4	287	298	287	284	3.45
5	303	301	308	305	3.42
6	296	301	298	266	4.02
7	292	289	281	285	3.73
8	290	293	287	282	3.66

FLOWER STRUCTURE AND THE INFLUENCE OF DAYLENGTH ON FLOWER INITITATION OF SERRURIA FLORIDA KNIGHT (PROTEACEAE)

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Abstract

Reproductive development of Serruria florida commences during March with the terminal inflorescence slowly increasing in weight while the apical meristem is producing peduncular bracts (pb). Following pb initiation the growth rate increases, the apical meristem enlarges and produces 30-40 bracts of which 10-15 abort shortly afterward. Incandescent light applied from 5 March 1988 from 6 pm to 8 am prevented flower formation indicating that the natural short days of winter are required for flowering.

Introduction

Serruria florida is commercially grown as cut flower under winter rainfall conditions in the Republic of South Africa. The major problem in marketing the crop is a very short flowering period. The development of the genus Serruria as flowering pot plants is also hindered by an inability to control its flowering time. The plants grow vegetatively during spring and summer. Reproductive development commences during autumn after extention growth has terminated, and flowers open during July and August.
The purpose of this study was to investigate the morphological development of the conflorescence and the role of photoperiod in induction of flowering.

Materials and methods

Plant material. Serruria florida plants grown in a commercial plantation were used. The plants were 4 years old and were propagated from seed. The plants were clean cultivated and not irrigated or fertilized. The mean annual rainfall for the area is 600-700 mm. The plants were interplanted between Protea cv. Carnival and spaced 1,5 m in the row and 3,5 meters between rows.

Conflorescence structure. Twenty shoots were randomly selected on 13 July and the following determined: the

Evaluation of pre-emergence herbicides on four proteaceous species

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Sequential applications of three pre-emergence herbicides were made to four species of newly planted proteas. Herbicides were applied at three-month intervals over a two-year period. Protea trunk diameter growth in response to herbicide treatments and two types of synthetic mulch were recorded after a one-year establishment period. *Protea neriifolia* R. Br. growth was significantly reduced by oryzalin at 6.7 kg a.i. ha^{-1}. *Banksia menziesii* R. Br. growth was reduced in untreated plots where weeds were removed by hoeing: growth of all other proteas was improved with weed removal. Growth of all other *Protea* species was unaffected by herbicide applications. Synthetic mulches of woven black polypropylene and a polyester spunbound fabric did not adversely affect protea growth. However, the polyester mulch collected soil, providing a surface for weed-seed germination and growth through the fabric. All herbicides tested provided adequate weed control, but oryzalin provided only marginal control of a leguminous weed, *Medicago truncatula* Gaertn.

Keywords: Protea; Herbicides; Weed control; Growth; Mulches; Hawaii

Protea is a commonly-used collective term applying to several genera of the family Proteaceae used for cut flowers. Proteas are indigenous to South Africa and are grown commercially in Australia, Israel, Southern California, Hawaii and New Zealand. In 1986, sales of proteas (cut flowers) from Hawaii amounted to 9.5 million U.S. dollars (Davies, 1986).

Weed control is an important problem with proteas due to their shallow proteoid roots (dense surface roots used for nutrient absorption) which are easily damaged by hand-hoeing. In Hawaii, Nishimoto (1975) reported that triflurualin (1.2 and 2.2 kg a.i. ha^{-1}), nitrofen (4.5 and 8.9 kg a.i. ha^{-1}), oxadiazon (3.4 and 6.7 kg a.i. ha^{-1}), diphenamid (6.7 and 13.5 kg a.i. ha^{-1}) and DCPA (22.4 kg a.i. ha^{-1}) caused only slight injury to *Protea eximia* (Salisb. *ex* Knight) Fourcade, *Leucadendron discolor* Buek *ex* Phillips & Hutchinson and *Leucospermum cordifolium* (Salisb. *ex* Knight) Fourcade when evaluated four months after application. In a subsequent experiment, dichlobenil at 4.5 and 8.9 kg a.i. ha^{-1} caused only slight injury to *P. eximia*, *L. cordifolium* and *Banksia menziesii* R. Br. Simazine at 8.9 kg a.i. ha^{-1} caused slight to moderate injury to *B. menziesii* and *P. eximia* but had negligible effect on *L. cordifolium*. Ametryn (4.5 and 8.9 kg a.i. ha^{-1}) and diuron (3.4 and 6.7 kg a.i. ha^{-1}) caused moderate injury to *P. eximia*, *L. cordifolium* and *B. menziesii*. EPTC, applied as a pre-plant incorporate treatment, caused moderate to severe injury on *P. eximia* and *L. discolor* at 3.4 and 6.7 kg a.i. ha^{-1}, respectively. EPTC at 6.7 kg a.i. ha^{-1} killed *L. cordifolium*. In New Zealand, Cox (1985) reported that dichlobenil at 8 kg a.i. ha^{-1} damaged *Leucospermum* and *Leucadendron* species but did not affect *Protea* species. A herbicide mixture of terbuthylazine and terbumeton (5 kg a.i. ha^{-1}) and simazine (3 kg a.i. ha^{-1}) damaged *Leucospermum*. Oxadiazon and oxyfluorfen (rates not reported) caused negligible effects on all species. The conflicting reports of phytotoxicity with dichlobenil between Hawaii and New Zealand could be attributed to the differences in soil organic matter content. Soil organic matter at the Hawaiian site was 13% and sandy soils were used in New Zealand (Nishimoto, personal communication). In California, Besemer and Elmore (1987) evaluated several pre-emergence herbicides on a one-year-old planting of *Protea neriifolia* R. Br. ('Pink mink'). Application of oxyfluorfen (1.1 and 2.2 kg a.i. ha^{-1}), oryzalin (4.4 and 8.9 ka a.i. ha^{-1}), simazine (2.2 kg a.i. ha^{-1}) and oxadiazon (2.2 and 4.4 kg a.i. ha^{-1}) did not cause any phytotoxic effects. However, oxadiazon did appear to be delaying protea growth.

In all these experiments, crop growth was evaluated after a single herbicide application. Weed control in commercial operations requires sequential herbicide applications to maintain a weed-free area around the base of the plant. The objective of this research was to determine growth and weed control in response to sequential applications of pre-emergence herbicides during the establishment of newly planted proteas.

Materials and methods

The experiment was conducted at the University of Hawaii's Kula Branch station on the island of Maui in the Hawaiian island chain. Elevation of the farm was 914 m with an average annual rainfall of 726 mm. The soil type was a Kula loam (subgroup, Typic Eutrandepts) with organic matter content of 13.3% at a pH of 6.6.

An experimental unit encompassed an area of 1.5 × 4.5 m and contained four plants. Treatments were replicated four times, using a randomized complete block design. Each experimental unit contained one seedling or rooted cutting (one-year-old) of each of the following proteas: *Leucadendron* hyb. cv. 'Safari Sunset', *Leucospermum cordifolium* (Salisb.

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eed control rating 90 days after the third of a sequence of herbicide applications to protea plants

Treatment	Rate (kg ha^{-1})	Weed control ratings[a] by species					
		A	B	C	D	E	F
Weeded control	–	0.0	0.0	7.5	0.0	0.0	2.5
Unweeded control	–	0.0	2.5	2.5	0.0	0.0	7.5
Oxadiazon	4.5	9.0	9.8	10.0	7.8	7.5	9.5
Oxyfluorfen 2% + Oryzalin 1%	3.4	8.8	10.0	10.0	7.5	7.8	8.8
Oxyfluorfen 2% + Oryzalin 1%	6.7	8.8	10.0	10.0	7.5	8.3	9.3
Oxyfluorfen 2% + Oryzalin 1%	10.1	10.0	10.0	10.0	7.5	8.8	9.5
Oryzalin	2.2	7.3	10.0	9.0	5.5	7.0	8.8
Oryzalin	4.5	8.8	10.0	8.8	5.3	8.3	10.0
Oryzalin	6.7	8.5	10.0	9.8	6.0	7.5	10.0
Black polypropylene woven ground cover (BPWGC)		10.0	9.3	10.0	10.0	10.0	10.0
Polyester geotextile fabric (PGF)		9.5	8.3	10.0	9.5	9.0	9.5
LSD (0.05)		1.3	2.2	3.3	1.4	1.2	3.3

[a] Zero rating, no control; 10, complete control
A, *Pennisetum clandestinum* Hochst. *ex* Choiv.; B. *Sonchus oleraceus* L.; C. *Ricinus communis* L.; D. *Medicago trunculata* Gaertn.; E. *Trifolium repens* L.; F. *Oenothera laciniata* Hill

ex Knight) Fourcade cv. 'Pincushion', *Banksia menziesii* cv. 'Pink Frost Banksia' and *Protea neriifolia* cv. 'Pink Mink'. The first herbicide application was made on 18 April 1986 with subsequent applications 90, 182, 267, 357, 448, 535 and 640 days later. All plots except the unweeded check were weeded before each sequential herbicide application. The herbicides applied were a granular formulation of oxadiazon (2%) at 4.4 kg a.i. ha^{-1}, a granular formulation of oxyfluorgen (2%) + oryzalin (1%) at 3.3, 6.7 and 10.1 kg a.i. ha^{-1}, and oryzalin at 2.2, 4.4 and 6.7 kg a.i. ha^{-1}. Oryzalin was applied with a CO_2-powered sprayer using Spraying Systems Co. 8004 LP nozzles delivering 350 l ha^{-1} at a pressure of 125 kPa. Herbicide applications were made to weed-free plots with sprays directed to the bases of plants. Granular herbicides were carefully applied to avoid retention by protea leaves. Control treatments consisted of plots hand-hoed at each herbicide re-application date and unweeded plots. Two synthetic mulches, a black polypropylene woven ground cover (BPWGC) and a gray, spunbound polyester geotextile fabric (PGF) were compared as weed control treatments. Both materials allow for the passage of air and water. Irrigation was provided as needed with a single drip-irrigation line placed in the centre of each plot. No fertilizers were applied at any time during the course of the experiment. Pesticides sprays for insects and diseases were applied as needed for commercial production.

Visual ratings of weed control were made before each herbicide application. A weed control rating made 267 days after planting (90 days after the previous herbicide application) will be reported as representative of herbicide performance throughout the experiment. Weed control was rated on a scale 0–10. A zero rating indicates weed control equal to the untreated plots (hand-weeded controls) and 10 indicates complete weed control; a rating of 7 is the minimum for commercially acceptable weed control. At 357 days after the start of the experiment, trunk diameter at 100 mm above the soil level was recorded and marked for subsequent measurements. A final diameter was taken 367 days later (724 days after start). The increase in trunk diameter between these two dates was used as a measure of protea growth.

Weed control ratings and increase in trunk diameter were subjected to an analysis of variance. The data presented for protea growth is the mean of four plants of each species.

Results and discussion

The mean of weed control ratings, made 90 days after the third herbicide application, are contained in Table 1. The predominant weeds rated for control were: *Pennisetum clandestinum* Hochst. ex Choiv. (kikuyugrass), *Sonchus oleraceus* L. (annual sowthistle), *Ricinus communis* L. (castorbean), *Medicago truncatula* Gaertn. (medic), *Trifolium repens* L. (white clover) and *Oenothera laciniata* Hill (cutleaf evening primrose). The weeded control was not maintained in a weed-free condition throughout the experiment – rather, weeds were removed at the time of each herbicide reapplication, thus permitting regrowth. The apparent control of *O. laciniata* in the unweeded control is due to competition from other weeds, primarily *P. clandestinum*.

The data indicate that commercially acceptable weed control was obtained with herbicides and mulch treatments on all weed species present, with the exception that all rates of oryzalin showed weakness in controlling *M. truncatula*. *T. repens* was marginally controlled at the low rate of oryzalin. Near perfect weed control was obtained with oxadiazon and with oxyfluorfen (2%) + oryzalin (1%).

By the end of the experiment, the composition of the weed spectrum had changed somewhat. Although data are not presented, it was clear that *Bidens pilosa* L. (Spanish needle) was not being controlled by the oxadiazon. All other herbicide treatments maintained control of this weed throughout the experiment.

Weed control by the synthetic mulches was mixed. The BPWGC controlled all weeds for the duration of the experiment. PGF permitted soil accumulation, allowing weed-seed germination and growth. Several weeds were also found sprouting from the PGF even though no soil was present. The cost of PGF and the accumulation of water-carried soil on it make it an unacceptable mulch for weed control.

Table 2 The increase in trunk diameter of protea plants in response to weed control treatments. Growth measurements for 367 days following a 1-year establishment period

Treatment	Rate (kg ha^{-1})	Increase in trunk diameter (mm)			
		A	B	C	D
Weeded control	–	19	18	20	24
Unweeded control	–	5	9	5	18
Oxadiazon	4.5	34	24	20	23
Oxyfluorfen 2% + Oryzalin 1%	3.4	24	24	21	27
Oxyfluorfen 2% + Oryzalin 1%	6.7	30	24	22	33
Oxyfluorfen 2% + Oryzalin 1%	10.1	32	25	22	15
Oryzalin	2.2	33	21	18	26
Oryzalin	4.5	29	24	22	16
Oryzalin	6.7	29	21	22	9
Black polypropylene woven ground cover (BPWGC)		38	25	19	31
Polyester geotextile fabric (PGF)		31	25	15	23
LSD (0.05)		11	5	11	14

A, *Banksia menziesii*; B, *Leucadendron* hybrid; C, *Leucospermun cordifolium* (Salisb. *ex* Knight) Fourcade; D, *Protea neriifolia*

The data of trunk growth are contained in Table 2. Applications of oryzalin at 6.7 kg a.i. ha^{-1} and the highest rate of oxyfluorfen + oryzalin were the only herbicide treatments to cause a growth reduction in *Protea neriifolia*. During the course of the experiment, no visible adverse affects of the herbicide or mulches were observed on any species. The reduced growth in the weeded control for *Banksia menziesii* in comparison with all other herbicide and mulch treatments (with the exception of oxyfluorfen + oryzalin at 4.4 kg a.i. ha^{-1}) demonstrates the sensitivity of this species to hoeing for weed removal. Even with careful hoeing, it was impossible to remove weeds without damaging some surface proteoid roots. Growth in all unweeded plots was significantly reduced in all species with the exception of *P. neriifolia*.

Conclusion

Commercially acceptable weed control can be obtained in proteas with granular formulations of oxadiazon, oxyfluorfen + oryzalin and sprays of oryzalin. Leguminous weeds may be marginally controlled with oryzalin spray applications. Protea growth is not affected by sequential applications (approximately 3-month intervals) of oxadiazon. Sequential herbicide applications, containing oryzalin, should not exceed 3 kg a.i. ha^{-1} in plantings of *P. neriifolia*. Woven black polypropylene ground cover suppresses weeds and is superior in this regard to polyester geotextile fabric which accumulates soil and allows weed growth through it. Proteas under synthetic mulches (as described here) grew normally and were free of weed competition.

References

Besemer, S.T. and Elmore, C.L. (1987) Weed control in *Protea neriifolia*: "Pink Mink", *Protea News* **6** 9–11

Cox, T.I. (1985) Tolerance of proteaceous species to residual herbicides (abstract #08504), in: *Annual Report 1985/86 – Southern North Island*, New Zealand Agricultural Research Division, Palmerston North

Davies, A.R. (1986) Statistics of Hawaiian agriculture, *Hawaii Agricultural Statistics Service*, Honolulu

Nishimoto, R.K. (1975) Weed control in established Proteaceae with soil residual herbicides, *Protea Workshop Proceedings*, December 12 1975, University of Hawaii, Cooperative Extension Service Miscellaneous Publication **139**

Post harvest disinfestation of export proteas

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Summary

A comparison of treatments for the post harvest control of spiders and earwigs in export proteas showed that a combination of permethrin (0.036 g a.i. m^{-3}) and dichlorvos (0.038 g a.i. m^{-3}) was more satisfactory than either permethrin alone or a combination of pyrethrins (0.008 g a.i. m^{-3}) and dichlorvos. Subsequent commercial use of the permethrin and dichlorvos treatment resulted in no detection of spiders or earwigs in proteas exported to Japan and U.S.A.

Introduction

Export of proteas, especially to Japan, has been hampered by the presence of live insects and spiders which has resulted in costly fumigation treatments at the port of entry and loss of quality due either to the treatment or to delays (Brinson 1987).

Most protea exporters conduct disinfestation treatments before packaging. An accepted treatment in South Africa has been the use of dichlorvos, either as a vapour treatment or by spray injection (Meynhardt 1976). More recently, methyl bromide was recommended, although some phytotoxicity was shown and not all insects and mites were completely controlled (Wit and van de Vrie 1985).

Fumigation tests in Australia with methyl bromide, carbon dioxide, nitrogen, sulphur dioxide, pyrethrins and dichlorvos, confirmed that methyl bromide reduced vase life of proteas and showed that a combination of pyrethrins and dichlorvos was the most suitable treatment (Maughan 1986). The combination was effective in killing a high percentage of the major pest species and did not affect vase life. However, some live arthropods, including spiders, could occasionally be found in export flowers and this did not satisfy the strict requirements of the Japanese market

The present work was aimed at finding a more satisfactory treatment for the control of the black house spider *Badumna insignis* (L. Koch) and the European earwig *Forficula auricularia* L., the most prevalent arthropods in cut proteas in Victoria (Brinson, pers. comm). The pyrethroid permethrin was tested because of its registration as a household space treatment for a range of pests including spiders.

Materials and methods

Tests were conducted in the coolroom at Protea Australia, Silvan, from October until December, 1987. The room had a volume of 32 m^3 and contained two-tiered wooden shelving on each side and in the centre. It was sealed and fitted with an exhaust fan. A thermostatically controlled fan heater was used for temperature control. An average temperature of 20°C (range 18-22°C) was maintained during the treatment time of two hours. The relative humidity varied in each test between means of 70 and 87%. Following treatment, the room was exhausted for half an hour (tests for dichlorvos using a Dräger detector showed that this was sufficient to remove all traces of the gas).

The treatments tested were (a) pyrethrins + dichlorvos (b) permethrin and (c) permethrin + dichlorvos each replicated five times. They were completely randomised over the two month period of the trial. Pyrethrins (as C.I.G. Pestigas$^{(R)}$) and dichlorvos (as C.I.G. Insectigas-D$^{(R)}$) came in gas cylinders and were introduced to the room through a nozzle giving a gas flow of 6 g sec^{-1}. Dosage was controlled by means of an automatic timer. A dosage of 0.008 g a.i. m^{-3} was used for pyrethrins and 0.038 g a.i. m^{-3} for dichlorvos. Permethrin (as Pea Beu Control) came in an aerosol pressure can with a lockable nozzle which allowed the contents to be exhausted. This gave a dosage of 0.036 g a.i. m^{-3}.

All tests were conducted on buckets of cut protea flowers. Space was made on the lower shelves (92 mm above the floor) for three large (69 x 59 x 8 mm) plastic trays on each of which was seated a bucket of eight flowers of *Protea magnifica* L. in water. These were located on each side of the room and in the centre. The sides of the trays were coated with Fluon (polytetrafluoroethylene) to prevent the spiders and earwigs from escaping.

The *P. magnifica* flowers contained some spiders (0-3 per bucket) and earwigs (0-8 per bucket) but they were insufficient and too variable for significant results. Therefore, 15 spiders and 15 earwigs were added to each test bucket of flowers and allowed to settle in for one hour before fumigation. These arthropods were obtained from a protea plantation on the morning prior to treatment.

Following treatment, all arthropods were collected from the test flowers, the trays and the water in the buckets and held separately in crumpled tissue in plastic vials with wire mesh lids for assessment of mortality after 24 and 48 hours. Similar numbers of untreated arthropods were also held in vials for assessment of control mortality. Analysis of variance procedures and a generalised linear model using a binomial error distribution were used to compare the three treatments.

Results

Both analyses gave similar interpretations and results from the analysis of variance are presented here.

The numbers and mortality of spiders and earwigs remaining in the flowers after each treatment are shown in Table 1. There was no significant difference between the treatments in the percentage of spiders remaining in the flowers or in their mortality after 24 h. There was a significantly lower ($P < 0.01$) percentage of earwigs in the flowers after the permethrin treatment compared with the other two treatments but the percentage mortality of these earwigs was also significantly lower ($P < 0.01$).

The mortality of all spiders and earwigs i.e. from the flowers, trays and buckets, is shown in Table 2. Both the permethrin and permethrin + dichlorvos treatments gave a significantly higher ($P < 0.01$) percentage mortality of spiders than the pyrethrins + dichlorvos treatment after 24 and 48 h. There was no significant difference between pyrethrins + dichlorvos and the other two treat-

Table 1. Mean percentage (dead + alive) and mortality (24h) of spiders and earwigs remaining in flowers after treatment.

Treatment	% remaining in flowers (dead + alive)		% mortality within flowers	
	Spiders	Earwigs	Spiders	Earwigs
Pyrethrins + Dichlorvos	36.1	14.3	80.6	83.2
Permethrin	24.3	4.7	94.2	41.7
Permethrin + Dichlorvos	28.5	18.2	88.0	96.8
L.S.D. (P=0.05)	20.5	7.0	20.8	19.3
(P=0.01)	28.7	9.7	29.2	27.5

Table 2. Mean percentage mortality of all spiders and earwigs at 24 and 48 h following treatment.

Treatment	Percentage Mortality			
	Spiders		Earwigs	
	24 h	48 h	24 h	48 h
Pyrethrins + Dichlorvos	83.7	87.3	94.6	96.4
Permethrin	96.0	98.8	93.3	93.3
Permethrin + Dichlorvos	96.7	97.5	99.1	99.4
L.S.D. (P=0.05)	7.8	7.2	4.7	3.8
(P=0.01)	10.9	10.1	6.6	5.4
Control^	1.9	2.7	0	0.3

^ Not included in analysis

ments in the percentage mortality of earwigs, but permethrin + dichlorvos gave significantly higher mortality than permethrin at 24 h ($P < 0.05$) and 48 h ($P < 0.01$).

Discussion

Dichlorvos, as a 12 hour vapour treatment or an individual flower injection, was found in South Africa to give effective control of most insects (Meynhardt 1976). It was not tested on its own in these trials because of its relative ineffectiveness against spiders (Maughan 1986). Maughan's best treatment of pyrethrins + dichlorvos was, therefore, used as the standard for spider and earwig control. Her recommendation was for a treatment time of six hours but, as Protea Australia were obtaining reasonable results with pyrethrins + dichlorvos using a two hour treatment time, this was used in these tests.

A temperature of 20°C was chosen for the tests as a balance between a temperature high enough to give effective pest control over the relatively short treatment time of two hours but low enough not to damage the flowers. As it was, some stress may have been placed on the flowers in this experiment because the treatment room was also the coolroom and had to be heated from 5 to 20°C before treatment. Despite this, there was no visual evidence of shortened vase life either in the flowers used in the tests or in the export shipments by Protea Australia. In practice, however, it would be advisable for growers to fumigate when flowers are first delivered to the packing shed, before cooling them prior to packing.

Protea magnifica flowers were used in these tests because they were considered to be more infested with spiders and earwigs than most other species, due to the size and shape of their inflorescence. The compactness of the floral bracts also makes it more difficult to reach pests with fumigants or aerosols. The results obtained for *P. magnifica* should, therefore, be applicable to other *Protea* species and may be better for less compact flower heads.

The arthropods remaining in the flowers were assessed separately because preliminary tests had indicated that although permethrin did not give 100% control of earwigs, it did tend to drive them out of the flowers. The current tests showed that there was a significantly lower percentage of earwigs in the flowers after the permethrin treatment. However, the percentage mortality of these remaining earwigs was also significantly lower than in the other two treatments.

When the total mortality of spiders and earwigs from the flowers, the trays and the buckets was considered, the permethrin + dichlorvos treatment was significantly better than permethrin for earwig control and significantly better than pyrethrins + dichlorvos for spider control. Although complete mortality of spiders and earwigs was not achieved, the high mean levels attained (97.5% and 99.4% respectively after 48 h) should be sufficient to ensure that none are detected in export produce. This is supported by the fact that Protea Australia used the two hour permethrin + dichlorvos treatment for all export flowers to Japan and U.S.A. in the 1987/88 summer season and no spiders or earwigs were detected by the importers.

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References

Brinson, A.J. (1987) Protea growers annual conference. *Protea National* 1 (9), 11-15.

Maughan, J.P. (1986) Post harvest treatment of protea cut flowers to eradicate arthropods. *Protea National* 1 (3), 13-17.

Meynhardt, J.T. (1976) Pests and diseases of proteas. In "Flowers, ornamental shrubs and trees" Series B. 6. Horticultural Research Institute, Pretoria, South Africa, pp. 1-4.

Wit, A.K.H. and van de Vrie, M. (1985) Fumigation of insects and mites in cut flowers for post harvest control. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent* 50 (2b), 705-12.

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A PRELIMINARY STUDY OF THE NITROGEN NUTRITIONAL STATUS OF MEMBERS OF THE SOUTH AFRICAN PROTEACEAE

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Abstract

An investigation of the free amino compounds of *Leucadendron xanthoconus* and *Protea lepidocarpodendron* leaves indicate very low concentrations of these compounds present. An analysis of the xylem sap of *L. xanthoconus*, *P. lepidocarpodendron*, *P. laurifolia* and *Brabeium stellatifolium* also reveals very low levels of nitrogen transporting compounds present, the spectrum of these compounds being dominated by nitrate and ammonium ions, and glutamine. Feeding experiments with KNO_3 and NH_4Cl indicate poor utilisation of additional nitrogen supplies by *L. xanthoconus* shoots, particularly in the case of nitrate feeding. ^{15}N feeding experiments do, however, show limited nitrate reduction capability in the leaves of *L. xanthoconus*.

Uittreksel

'N VOORLOPIGE STUDIE VAN DIE STIKSTOF-VOEDINGSSTATUS VAN LEDE VAN DIE SUID-AFRIKAANSE PROTEACEAE

'n Ondersoek van die vrye aminoverbindinge van *Leucadendron xanthoconus* en *Protea lepidocarpodendron* blare vertoon die aanwesigheid van baie lae konsentrasies van hierdie verbindinge. Oplossings van die xileemsap van *L. xanthoconus*, *P. lepidocarpodendron*, *P. laurifolia* en *Brabeium stellatifolium* besit ook 'n baie lae konsentrasie van stikstofvervoerverbindinge, met nitraat- en ammoniakione, en glutamien as die hoofkomponente. Voedingseksperimente met KNO_3 en NH_4Cl vertoon swak benutting van sulke bykomende stikstofbronne deur uitspruitsels van *L. xanthoconus*, veral in die geval van nitraatvoeding. ^{15}N voedingseksperimente het egter bewys dat die blare van *L. xanthoconus* oor 'n beperkte vermoë beskik om nitraat te reduseer.

Although the fynbos of the South Western Cape has been known to civilised man longer than any other vegetation type in Southern Africa, it remains one of the most poorly studied of these types, especially in relation to its ecophysiological relationships. This apparent neglect of the country's most spectacular flora is probably occasioned by three main factors: the complexity of the flora itself; the fact that the topography and soils outside the regions exploited early in the country's history are not favourable for agronomy; and the physical and chemical characteristics of the natural vegetation which make it unsuitable for feeding livestock or for other forms of commercial usage other than cut flowers.

The investigation to be described is a preliminary study of one aspect of fynbos nutrition: the nitrogen status of the family Proteaceae and its comparison with that of non-fynbos families, in an attempt to understand this feature of the

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nutritional strategy of the fynbos employed in coping with the severe nutritional limitations of the soils of their vegetational area.

In this study the following four aspects of the nitrogen nutrition of representative species of Proteaceae have been investigated:—

1. The spectrum of free nitrogen compounds found in the leaves of the species, the quantities of these compounds present and a comparison of these data with that of non-fynbos plants.
2. The spectrum and relative quantities of the translocatory nitrogen compounds responsible for conveying nitrogen from soil to leaf via the xylem stream of the root and stem, and a comparison of these data with relative data obtained for non-fynbos representative species.
3. The effect of feeding additional supplies of nitrate or ammonium ions to the shoots of these plants to ascertain whether these supplies can be utilised and, if so, their influence on nitrogen metabolism in the leaf.
4. The nitrate reducing properties of the leaves of these plants and an assessment of the role of nitrate in leaf nitrogen nutrition.

Plant Material and Methods

Four species of Proteaceae, *Protea lepidocarpodendron* L., *Protea laurifolia* Thunb., *Leucadendron xanthoconus* O. Ktze. and *Brabeium stellatifolium* L. were investigated. *Protea lepidocarpodendron* and *Leucadendron xanthoconus* were growing at 300 m on the N.W.-facing slopes of Orange Kloof, Table Mountain, in soil derived from Table Mountain Sandstone. *Protea laurifolia* and *Brabeium stellatifolium* were growing respectively on the N.E.-facing slopes and the river valley floor of Happy Valley, Bains Kloof in Table Mountain Sandstone derived soil at an altitude of 630 m. Sampling was carried out during April and May 1976, i.e. in late autumn.

Leaf Sampling and Amino Compound Extraction. When leaf samples were removed from plants in the field, they were immediately killed and frozen by immersing them in liquid nitrogen. These frozen samples were returned to the laboratory where known weights of leaf material were homogenized in cold 80% ethanol (100 mℓ 80% ethanol for 5 g leaf material) using an Ultra Turrax homogenizer. The preparations were allowed to extract for 24 h at 0 ° in a sealed flask with occasional shaking, filtered and reduced in volume to 10 mℓ under an airstream.

Xylem Sap Extraction. Xylem sap was extracted from the plants by cutting off leafy twigs in the field and applying pressure to them inside a Scholander Bomb. The xylem sap extruded from the cut surface of the twig stem was collected and frozen immediately.

Soil Nitrate Analysis. Soil samples were collected in plastic bags from under the experimental plants and frozen immediately by immersing them in liquid nitrogen, thus killing the soil bacteria. These were transported to the laboratory,

dried at 30 °C and extracted by shaking in distilled water (1:1 weight/volume) for 30 min. Nitrate analysis was performed on soil extracts and xylem sap using an Orion Nitrate Probe coupled to an Orion Model 701 pH Meter.

Amino Compound Analysis. Amino acid and amide content estimates of leaf extracts and xylem sap were made on a Beckman 120C Amino Acid Analyser using the lithium buffer methodology described by Lewis (1975).

Nitrate and Ammonium Ion Feeding. Nitrate ion and ammonium ion solutions were fed to detached shoots of *Leucadendron xanthoconus* in the field as follows:

Shoots were detached from plants at 10h00, their severed ends recut under water to prevent xylem airblocks and placed into beakers containing either a 200 μg N mℓ^{-1} KNO_3 solution or a 200 μg N mℓ^{-1} NH_4Cl solution. These shoots were allowed to photosynthesize in sunlight alongside the plants from which they had been detached for 8 hours, when they were frozen and extracted as described about. Reference shoots were removed from the plants for analysis at the beginning of the experiment and at the end of the 8-hour period.

A further experiment in which nitrate was fed continuously to detached shoots of *L. xanthoconus* for 24 h was performed in a "Conviron" controlled environment cabinet under constant light (16 000 lux), a temperature of 16 °C and a relative humidity of 80%.

The duplicated results of these feeding experiments are reported in Table 3.

^{15}N Isotope Studies on Nitrate Utilisation. In this experiment a leafy shoot of *L. xanthoconus* was fed a solution of 200 μg ^{15}N mℓ^{-1} K $^{15}NO_3$ for 24 h in a controlled environment cabinet under the conditions described above, to determine whether the shoots of this plant are able to reduce and utilise nitrate.

After extraction, the amino compounds of the extract were separated on a 150 × 1,8 cm Beckman M-84 ion-exchange resin column and collected in a fraction collector. The amino compound samples were prepared for ^{15}N atomic emission analysis by Kjeldahl digestion and ammonia distillation. These analyses were performed on a Statron (Packard) atomic emission spectrophotometer following oxidation of the ammonia with sodium hypobromite by the method of Faust (1967).

Results and Discussion

Free Amino Compounds in the leaves of P. lepidocarpodendron *and* L. xanthoconus

The levels of free amino compounds occurring in these leaves are indicated in Table 1. Also shown in this table are the relative figures for a non-fynbos plant typified by *Datura stramonium* L. growing in fertilised agricultural soil near Cape Town. The same amino-compound extraction procedure was used for *Datura* as for the fynbos plants.

From this table it can be observed that the free amino acid and amide levels in the leaves of *P. lepidocarpodendron* and *L. xanthoconus* are extremely low when

TABLE 1

The concentrations (μmol g fr. wt.$^{-1}$) of the free amino compounds of the leaves of two members of the Proteaceae compared with *Datura stramonium*.

Amino Acids	*Leucadendron xanthoconus*	*Protea lepidocarpodendron*	*Datura stramonium*
Aspartate	0,13	0,16	2,67
Threonine	0,10	0,07	1,16
Serine	0,30	0,26	0,94
Asparagine	0,04	0,07	0,69
Glutamate	0,30	0,36	3,68
Glutamine	0,03	0,09	1,45
Proline	—	—	—
Glycine	0,26	0,25	0,08
Alanine	0,10	0,13	0,48
Valine	0,01	0,02	0,01
Cystine	—	—	—
Methionine	—	—	—
Isoleucine	0,03	0,03	0,26
Leucine	0,03	0,03	0,07
Tyrosine	0,02	0,02	0,13
Phenylalanine	0,02	0,03	0,04
Lysine	0,25	0,15	0,13
Histidine	0,10	0,05	0,04
Arginine	0,01	0,01	1,00

compared with those of *D. stramonium*, with serine, glycine and glutamate dominating the amino acid spectrum.

This low free amino compound level is an indication that the level of N metabolism activity in these fynbos plants is low in comparison with that of plants adapted to living under more favourable soil N nutritional conditions.

Free Amino Compounds in the Xylem Sap of P. lepidocarpodendron, P. neriifolia, L. xanthoconus *and* B. stellatifolium

Table 2 shows a quantitative comparison of the amino compounds, nitrate and ammonium ions being transported from root to leaf via the xylem sap in the four Proteaceae species with those of *D. stramonium*. In the Proteaceae species the extremely low concentrations of nitrogen compounds found in the xylem sap are immediately obvious, indicating the very restricted supply of nitrogenous nutrients to the shoot in comparison with a plant adapted to a more favourable nutritional environment.

In all four fynbos plants, glutamine is the dominant amino compound present in the xylem sap, reflecting the importance of this amide as a translocator of reduced nitrogen from root to leaf in many plants. The major utilisable nitrogen carrier appears to be ammonium ions.

The unreduced nitrogen supply to the leaf in the form of nitrate present in the xylem sap, is greater than the total reduced nitrogen supply (amino compounds +

TABLE 2

The free amino compounds of the xylem sap of 4 species of Proteaceae compared with *Datura stramonium*.

Amino Compounds	*Leucadendron xanthoconus* μmol N ml^{-1} xylem sap	*Protea lepidocarpodendron* μmol N ml^{-1} xylem sap	*Brabeium stellatifolium* μmol N ml^{-1} xylem sap	*Protea laurifolia* μmol N ml^{-1} xylem sap	*Datura stramonium* μmol N ml^{-1} xylem sap
Lysine	0,015	0,00	Trace	0,001	8,73
Histidine	Trace	0,001	Trace	0,002	6,42
Ammonia	0,198	0,174	0,030	0,054	23,91
Arginine	Trace	Trace	0,001	Trace	2,98
Aspartate	0,002	0,012	0,002	0,003	0,883
Threonine	Trace	0,004	0,001	Trace	1,310
Serine	0,003	0,010	0,002	0,001	0,180
Asparagine	Trace	0,003	0,002	Trace	3,297
Glutamate	0,002	0,008	0,002	0,002	2,063
Glutamine	0,012	0,040	0,030	0,014	25,31
Glycine	0,003	0,002	Trace	Trace	0,05
Alanine	0,001	0,005	Trace	0,001	0,190
Valine	—	—	—	—	4,80
Cystine	—	—	—	—	—
Methionine	—	—	—	—	—
Isoleucine	Trace	Trace	Trace	Trace	1,89
Leucine	Trace	0,001	Trace	—	2,47
Tyrosine	—	—	—	—	0,24
Phenylalanine	—	—	—	—	0,393
Nitrate N	0,800	1,200	0,300	0,400	173,0
Total N	1,036	1,463	0,370	0,478	258,12

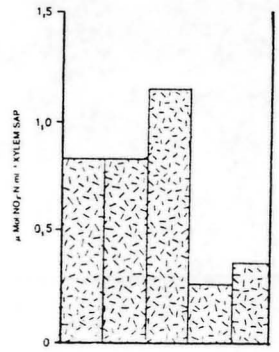

FIG. 1 (a)
Nitrate content of xylem sap of *Leucadendron xanthoconus* (LX) (two samples) *Protea lepidocarpodendron* (PL) *Brabeium stellatifolium* (BS) *Protea laurifolia* (PN)

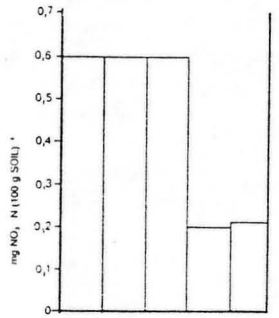

FIG. 1 (b)
Nitrate content of soils in which the plants were growing.

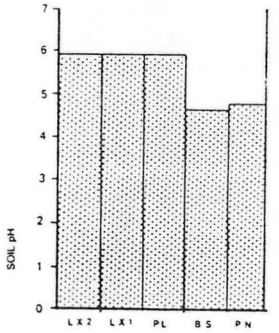

FIG. 1 (c)
pH of soils in which the plants were growing.

ammonium), but there is strong doubt as to whether this supply can be utilised by the leaf as the experiments to be described indicate.

The quantity of nitrate present in the xylem sap appears to be directly related to soil nitrate content and soil pH. Fig. 1 shows the correlation between xylem nitrate content, soil nitrate content and soil pH. The lower nitrate levels in the low pH Bains Kloof soils are probably a manifestation of the well known phenomenon of nitrifying bacteria inhibition in acidic soils.

The Effect of Nitrate and Ammonium Ion Feeding on Nitrogen Metabolism in Photosynthesizing shoots of L. xanthoconus

The results of the nitrate feeding experiments in which 200 μg N mℓ^{-1} was fed to photosynthesizing shoots via the xylem stream in the form of potassium nitrate are reported in Table 3. An inspection of this table shows no significant differences between the aminograms of experimental plants with their controls after a feeding period in the light of 8 h. After a 24 h feeding period there is a slight increase in the level of the N-incorporating amino acid, glutamic acid, indicating a small degree of nitrate ion utilisation by the shoots of *L. xanthoconus*.

The results of the ammonium feeding experiments in which 200 μg N mℓ^{-1} was fed to photosynthesizing shoots in the form of ammonium chloride are also shown in Table 3. An inspection of this table shows a considerable increase in the glutamine levels of the experimental plants compared with the control plants, indicating that the additional ammonium supply can to a large extent be incorporated into shoot metabolism. Glutamine has been shown to be an important contributor of reduced nitrogen to plant amino acid synthesis via the glutamate synthase pathway (Lee & Miflin, 1974) and is known to accumulate in plants receiving high nitrogen nutrition (Lewis & Berry, 1975).

Nitrate Reduction by Shoots of L. xanthoconus

The ^{15}N enrichment of three important free amino acids of the leaf of *L. xanthoconus* after feeding K$^{15}NO_3$ (99 atom % excess) at the 200 μg N mℓ^{-1} level for 24 h is shown in Table 4. These enrichments are extremely low for a 24 h feeding period and confirm the poor nitrate processing properties of *L. xanthoconus* shoots. It nevertheless appears that the shoots do have limited nitrate

TABLE 4
^{15}N enrichment of glutamate, aspartate and threonine in leaves of *L. xanthoconus* fed K $^{15}NO_3$ (200 μg ^{15}N mℓ^{-1}) through the xylem stream for 24 h.

	^{15}N CONC. (ATOM % EXCESS)
GLUTAMATE	6,3
ASPARTATE	5,0
THREONINE	1,6

TABLE 3

Effect of feeding KNO_3 and NH_4Cl solutions at 200 μg N ml^{-1} concentrations on free amino compound levels* (μmol g fr. wt.$^{-1}$) in leaves of *L. xanthoconus*.

	CONTROL (from shrub) (duplicate results)		KNO_3-fed		(duplicate results)		NH_4Cl-fed (duplicate results)	
	8 h	8 h	8 h	8 h	24 h	24 h	8 h	8 h
Aspartate	0,23	0,16	0,16	0,20	0,10	0,15	0,26	0,22
Threonine	0,04	0,05	0,06	0,07	0,05	0,04	0,07	0,12
Serine	0,15	0,07	0,19	0,25	0,04	0,12	0,40	0,30
Asparagine	0,02	0,02	Trace	Trace	Trace	Trace	0,03	0,15
Glutamate	**0,27**	**0,19**	**0,17**	**0,20**	**0,36**	**0,51**	**0,47**	**0,64**
Glutamine	**0,03**	**0,02**	**Trace**	**Trace**	**0,05**	**0,10**	**1,99**	**1,33**
Proline	—	—	—	—	—	—	—	—
Glycine	0,07	0,02	0,06	0,09	Trace	0,05	0,04	0,01
Alanine	0,05	0,01	0,06	0,08	0,20	0,20	0,19	0,04
Valine	—	—	—	—	—	—	—	—
Cystine	—	—	—	—	—	—	—	—
Methionine	—	—	—	—	—	—	—	—
Isoleucine	Trace	—	Trace	0,01	—	—	0,01	Trace
Leucine	—	—	—	0,01	—	—	0,01	Trace
Tyrosine	—	—	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—

*Recognised reduced-N accepting compound in bold type.

reducing properties which may be further induced after prolonged exposure to high nitrate concentrations.

CONCLUSIONS

If the species investigated in this report can be considered representative of the Proteaceae, it would appear that this family has adapted to the poor nutrient status of the soils in which it grows by evolving a low intensity nitrogen metabolism to correspond with a slow growth habit. It would also appear that the shoots of *L. xanthoconus* are unable to utilise to any great degree high levels of nitrogen supplied to them, especially if the nitrogen is provided in the form of nitrate. Other workers (Groves & Keraitis, 1977) have shown, indeed, that high levels of nitrogen (250 μg N $m\ell^{-1}$) and phosphorus feeding can prove fatal to *Banksia serrata*, an Australian member of the Proteaceae, confirming the restricted nitrogen processing potential of the family.

REFERENCES

FAUST, H., 1967. Probenchemie ^{15}N-markierter Stickstoffverbindungen im Mikro- bis Nanomolbereich für die emissionsspektrometrische Isotopenanalyse. *Isotopenpraxis* **3:** 100–103.

GROVES, R. H. and KERAITIS, K., 1976. Survival and growth of seedlings of three sclerophyll species at high levels of phosphorus and nitrogen. *Aust. J. Bot.* **24:** 681–690.

LEA, P. J. and MIFLIN, B. J., 1974. An alternative route for nitrogen assimilation in higher plants. *Nature, Lond.* **251:** 614–616.

LEWIS, O. A. M., 1975. An ^{15}N–^{14}C study of the role of the leaf in the nitrogen nutrition of the seed of *Datura stramonium* L. *J. exp. Bot.* **26:** 361–366.

LEWIS, O. A. M. and BERRY, M. J., 1975. Glutamine as a major acceptor of reduced nitrogen in leaves. *Planta* **125:** 77–80.