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Third International Protea Research Symposium

Editors
G.J. Brits
M.G. Watt

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THIRD INTERNATIONAL PROTEA RESEARCH SYMPOSIUM

Harare, Zimbabwe
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Convener
G.J. Brits

Editors
G.J. Brits
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Section for Ornamental Plants
Protea Working Group

Editors

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PREFACE

The International Protea Working Group (IPWG) presents research symposia on Proteaceae concurrently with international growers' conferences of the International Protea Association (IPA), at four-yearly intervals. For the continued and valued support of this concept the protea research fraternity wishes to acknowledge and thank the protea industry. The IPA this time hosted both the IPA 7th International Protea Conference and the 3rd Protea Research Symposium, in Harare, Zimbabwe. Apart from providing the framework for the combined meetings there was the gratifying participation by industry in our research sessions. The gathering of growers and scientists proved to be an excellent opportunity for the exchange of ideas, and it set the stage for continued joint meetings in the future. I wish to thank Mr. David Seaman, president of IPA and Chairman of the protea conference for making this possible.

The IPA has moreover sponsored the printing costs of this Acta proceedings. As convener of the research symposium I wish to offer sincere thanks to IPA for their generous support.

My appreciation goes to our panel of referees for their invaluable inputs into this volume.

To Dr Cobus Coetzee, the succeeding chairman of IPWG, I extend good wishes in promoting continued cooperation within our worldwide protean circle!

Gert J. Brits
Convener

THREATS TO THE CONSERVATION OF SOUTHWESTERN AUSTRALIAN PROTEACEAE

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Abstract

Southwestern Australia is a major genetic resource for the Proteaceae, with about 620 endemic species and subspecies. Their habitats are under threat in some areas from land clearing. The spread of *Phytophthora cinnamomi*, to which this family is particularly susceptible, has reached epidemic levels in the southern sandplains. Potential threats include increased fire frequency, invasion of post-fire vegetation by herbaceous weeds, reduced annual rainfall, and canker diseases. The levels of pollinators, granivores and herbivores, and burning in spring, are at present no cause for concern.

1. Introduction

Southwestern Australia is home to about 618 species and subspecies of Proteaceae, by far the greatest concentration in the world. This includes 7 genera in the Proteoideae, 7 in Grevilleoideae and one in Persoonioideae and four endemic genera (table 1). Many of these have outstanding value for amenity horticulture and floriculture.

Table 1. Proteaceae in the mediterranean climate of southwestern Australia - number of species per genus within subfamilies (compiled by B. Lamont).

Proteoideae		Grevilleoideae		Persoonioideae	
Adenanthos	30	*Banksia*	60	*Persoonia*	24
Conospermum	30	*Dryandra*	120◇	*Acidonia*	1*
Franklandia	2*	*Grevillea*	129#		
Isopogon	25	*Hakea*	93▲◇	*endemic	
Petrophile	38	*Lambertia*	10	◇some unpublished	
Stirlingia	5*	*Strangea*	2	# + 31 subspecies	
Synaphae	13*	*Xylomelum*	2	▲ + 4 subspecies	

This mediterranean climate region is now occupied by 2 million people and has been extensively cleared for housing, roads and farms. The vegetation is highly combustible and many species are susceptible to the widely distributed pathogen, *Phytophthora*

cinnamomi. The threats these and other constraints impose on the conservation of Proteaceae in this region are outlined here.

2. Loss of habitat

The Swan Coastal Plain is a major habitat for banksias and coincides to where most people live. By 1986, 55% of the banksia woodland had been cleared (Hopper and Burbidge, 1989). Only 7% of the original 281,000 ha is on conservation reserves. As Proteaceae tend to occur on the poorest soils (sands and laterites), areas in which they are abundant have been the last to be cleared. Opening up of the 'light lands' after World War II and especially parts of the northern and southern sandplains in the 1960's and 70's has led to direct threats and the spread of diseases and weeds. Rare species, such as *Grevillea scapigera* and *Dryandra mimica*, have become even rarer. Leigh and Briggs (1992) list 13 southwestern Australian Proteaceae threatened by agriculture and roadworks. They do not even mention the gazetted rare *Banksia cuneata* and *B. goodii* every population of which has either been lost or severely reduced in size (Lamont *et al.*, 1991a, 1993a).

3. Effects on flowering and seed production

Each species is predominantly pollinated by insects, birds or (rarely) mammals (Collins and Rebelo, 1987). A wide range of insects is used by any one species (Lamont, 1982; 1985). The introduced honey bee may be the major pollinator of some species (e.g. *Hakea trifurcata)* in some areas but not visit closely related co-flowering species nearby (e.g. *H. undulata)*. The honey bee appears to be an effective pollinator but its presumed impact on viability of native insect pollinators has not been studied. About 15% of Proteaceae in southwestern Australia are essentially bird-pollinated (Keighery, 1982; Hopper and Burbidge, 1986). A wide range of honeyeaters (Maliphagidae) is used (Collins and Spice, 1986; Lamont and Collins, 1988). No species is solely pollinated by small marsupials, although the honey possum is more active on *Banksia nutans* and *B. tricuspis* than honeyeaters (Wooller *et al.*, 1983; S. van Leeuwen, pers. comm.). Because of the continuing abundance and number of alternative animals available, there is as yet no evidence of pollinators limiting seed set except in the smallest remnant populations.

Population size may affect seed set. The larger the population of *Banksia goodii*, the greater the likelihood that individual plants will set seeds even though they flower the same time (Lamont *et al.*, 1993a, b). The critical size appears to be about seven plants as no seed set has been observed in smaller populations over 10 years. The explanation revolves around either a decline in pollinator (bird/ marsupial) visits or an increase in the likelihood of selfing in what is probably a self-incompatible species. The reason for small population sizes is clearing for agriculture and roads. Even the largest populations on reserves and uncleared farmland were once bigger and our data indicate that seed production per plant could be increased further. Clearly, the conservation status of this species would be improved by having fewer larger populations than many small populations summing to the same number of plants (Lamont *et al.*, 1993a).

Flowering and seed production may fluctuate greatly between years without any clear relationship with the current or previous year's weather conditions, granivore activity

or previous crop size (Cowling *et al.*, 1987; Lamont and Barker, 1988; Witkowski *et al.*, 1991). In many banksias, up to 70% of flower heads may be removed by larvae-seeking birds, up to 80% of barren cones may have been tunnelled by insect larvae and up to 40% of stored seeds may be destroyed by insect larvae (Abbott, 1985; Zammit and Hood, 1986; Lamont and Barker, 1988; Lamont and van Leeuwen, 1988; Lamont and Barrett 1988; Witkowski *et al.*, 1991). While this clearly results in greatly reduced seed release after fire, the long-term impact is unknown. If recruitment is a weighted lottery, then the greater the contribution of a species to total seeds available, the better. This is not as critical for resprouters as for non-sprouters. The black cockatoo actually acts as an efficient agent of biological control for *B. tricuspis* by identifying those flower heads that contain moth larvae and consuming them (Lamont and van Leeuwen, 1988; Lamont, 1993). Thus the conservation status of this bird could have implications for the long-term future of this rare species, although post-fire population turnover is usually negligible. *B. tricuspis* is restricted to Mt. Lesueur National Park which has been subject to a coal mining claim in the recent past, while population numbers of the black cockatoo have been declining steadily in living memory (D.A. Saunders, pers. comm.).

4. Effects of fire regime

Fire controls seed production and release, and population dynamics. If an intense (non-patchy) fire occurs before young plants have produced much seed then there is a high risk of local extinction. The longer the fire interval, the greater the likelihood of reaching the minimum number of seeds required for population restoration provided plant senescence has not been reached (Lamont *et al.*, 1991b). For *B. cuneata*, the optimum fire interval is about 20 years, assuming plant longevity is 45 years (Burgman and Lamont, 1992). Population size will gradually approach zero for increasingly longer intervals than the optimum (due to plant deaths and thus seed wastage) and increasingly shorter intervals (due to insufficient seed storage). We have estimated a minimum fire interval of 10 years for a number of other non-sprouting banksias (Cowling *et al.*, 1990).

For most resprouters, there is complete recovery of adults after fire (Enright and Lamont, 1992; Lamont *et al.*, 1993b). However, recovery by *B. menziesii* depends on location: over 20% of adults die in the drier northern end of its range, while 5% die in the southern end (Whitten *et al.*, 1993). We attribute greater death rates in the north to the fact that plants are smaller and more likely to be completely burnt (Cowling and Lamont 1985) and a recent increase in fire frequency associated with greater human activity following establishment of the Brand Highway in the 1960's. Mallee form *B. menziesii* is more fire tolerant than the tree form while initially unburnt plants are likely to develop into small trees in the north. Another potential problem is the extended time it usually takes for young plants to become fire-tolerant. For species taking more years to become fire-tolerant than the average fire interval, and with incomplete survival of fire by adults, the population is likely to decline gradually.

Colonization by Europeans has generally led to an increase in fire frequency (Lamont and Downes, 1979; Anderson and Muir, 1981; Muir, 1985). A combination of seven lightning-caused, prescribed and escaped fires has resulted in nine of our ten 20 x 20 m permanent plots scattered along 5 km of Mt. Adams Road with

Proteaceae-dominated scrub-heath being burnt twice between 1983 and 1993 (Cowling and Lamont, 1987; Enright and Lamont, 1992). The non-sprouter *B. hookeriana* has now been eliminated from two of these. In contrast, numbers of the resprouter *B. attenuata* have increased with each fire, but *B. menziesii* has decreased. Ironically, landscape fragmentation has resulted in many community remnants which are now rarely burnt (Muir 1985). One patch of banksia woodland at Lake Indoon has escaped fire for about 40 years. Such old stands provide an essential baseline for fire studies and modelling, for they show the way non-sprouting plants senesce and die (Burgman and Lamont, 1992), what happens to seed production over an extended time, and whether interfire establishment starts to become significant. We have only encountered senescence in the usual sense in *B. coccinea* (Witkowski *et al.*, 1991; Lamont, 1992) and it is possible that even this was induced by canker disease. Plants are usually still growing vigorously at 30 years (Muir, 1985; Lamont *et al.*, 1991a) except where main branches break off large exposed plants. Thus it is most unlikely that lack of fire will ever be the cause of a population's demise.

Fire intensity and season are also relevant. They are usually confounded - spring fires are rarely as intense as those in autumn. Weakly serotinous species release their seeds spontaneously in late autumn (Abbott 1985). The great majority of species in *Banksia, Dryandra, Hakea, Lambertia, Petrophile* and *Xylomelum* however retain most of their seeds on the plant for several years, relying on flame heat for follicle or cone opening (Lamont *et al.*, 1991b). Ten years is a typical lifespan for banksia seeds retained in their follicles. Unlike *Hakea, Isopogon, Lambertia* and *Petrophile*, plant death causes only a minor increase in the background rate of seed release in *Banksia, Dryandra* and *Xylomelum*. However, since pre-fire dead plants are usually incinerated when fire does occur (Lamont and Barker, 1988; *B. brownii* - Lamont and H. Galea unpubl.), the seeds remaining are wasted. Mass death of stands of Proteaceae due to drought, waterlogging, *Phytophthora* or canker disease followed by intense fire could lead to local losses or increased rarity of species.

If a fire is patchy or passes beneath the canopy of the plants then the critical temperature for seed release may not be reached. This will further delay fire-induced seed release from those plants until the vegetation can carry another (more intense) fire. Spring and patchy fires therefore result in less seed release and fewer post-fire seedlings (Cowling and Lamont, 1987; Enright and Lamont, 1989a; Lamont *et al.*, 1993c). But population maintenance may not depend on maximum post-fire seed release as discussed below.

5. Effects on seedling establishment

The period between seed release and seedling establishment is critical in the life cycle of Proteaceae. Most of the seeds fall beneath the crown where they remain or get redistributed into litter microsites by wind or sometimes surface water (Lamont *et al.*, 1993c). We have shown that seeds sitting on the surface for an extended period are likely to die from summer heat (Cowling and Lamont, 1987; Enright and Lamont, 1989) or granivory (Cowling and Lamont, 1987; *Banksia brownii*, B. Lamont and H. Galea, unpubl.). We used these observations to support the opinion that autumn fires are more beneficial than (management-preferred) spring fires – where germination is delayed until the following winter (Cowling *et al.*, 1990).

However, our more recent work indicates that this conclusion was premature: the ecosystems are so finely buffered that the eventual outcome of spring/mild fires is usually little different from that of autumn/intense fires. To begin with, most sites are wind-prone after fire and seeds of banksias, hakeas and petrophiles get buried quickly by sand or litter (Lamont *et al.*, 1993c). Most buried seeds germinate and competition for soil water in the litter patches is intense. The result is that greater proportions of seedlings in the litter than surrounding sand die (Lamont *et al.*, 1993b) as do those in autumn-fire litter than spring-fire litter (Enright and Lamont, 1989). After a patchy burn, competition of seedlings with surviving adults may lead to higher levels of death than in wildfire microsites (Lamont *et al.*, 1993c). But since some, already highly fecund, plants remain, the net effect may still favour the less intensely burnt site. Granivorous birds act swiftly after fire: flocks of black cockatoos settle on the larger plants to extract seeds exposed in the follicles (Cowling and Lamont, 1987) while almost all seeds of *B. brownii* placed on the soil surface in autumn burnt and unburnt sites were consumed by parrots within 3 – 4 weeks. This means that the timing of fires in relation to the opportunities for germination (germinants are not eaten by granivorous birds) is irrelevant to the extent of granivory, as germination takes at least 1 – 3 weeks even under ideal conditions.

Though our evidence is only based on symptoms, wingless grasshoppers appeared responsible for substantial loss of germinants at a number of our study sites throughout southwestern Australia – in some (but not all) burnt areas of small extent (2 – 5 ha) and especially unburnt sites (Cowling and Lamont, 1987). The cotyledons and first leaves were consumed on planted germinants of five *Banksia* species at Hopetoun on the south coast in a 21 year old stand but herbivory was negligible in experimental autumn and spring burnt sites nearby (B. Lamont and S. Connell, unpubl.). At Albany, older seedlings of two other *Banksia* species were uprooted by bandicoots or eaten by kangaroos at an unburnt site but not at a burnt site nearby. Lack of damage and 63% survival after two summers in the plots covered with aviary wire confirmed that the 2% survival in uncovered plots was the result of marsupial activity and not insects (Lamont *et al.*, 1993b). In one set of plots beside a farm, most germinants of *B. brownii* were killed by red-legged earthmites while another three sets in different habitats the same distance from the farm boundary were unaffected (B. Lamont and H. Galea, unpubl.).

The effects of summer drought can be equally devastating and fitful. It is as important as predation and inadequate seed release in explaining lack of seedling recruitment in unburnt stands (Lamont and Barker, 1988). After an experimental autumn fire, an estimated 13 860 seedlings of *Banksia cuneata* were recorded over June-October but by January only 11 survived (Lamont *et al.*, 1991a). Competition for water between seedlings of native species and annual weeds and lack of summer rain appeared to be responsible, as the last seedlings to die were in local depressions, especially if shaded or litter-filled. A field trial set up the following year showed that survival and plant size were greatest if they were in 'run-on' areas and watered over the summer. Our modelling of population dynamics of this species has shown that the outcomes are very sensitive to annual rainfall (Burgman and Lamont, 1992). Should rainfall continue to decline at an average rate of 4% per decade as it has done in the region over the last 70 years (Pittock, 1988) then the model shows there is over a 50% chance of our study population becoming extinct over the next 50 years.

Banksia goodii is restricted to a 37 x 16 km area with a 9 month wet season

(Prescott's formula). From transplant studies we showed it would survive at least four winters with an average 7 but not 6 month wet season (Lamont *et al.*, 1993a). Drought therefore limits where this rare species can be grown successfully in the attempt to increase its geographic range. Attempts to grow banksias in areas where they occur naturally but on unusual substrates has met with mixed success (Lamont *et al.*, 1989; Enright and Lamont, 1992a). Summer death is often most marked in the first year after fire. However, a mild summer with a substantial cyclone followed by a dry summer with temperatures up to 47 °C resulted in far more deaths of seedlings of four *Banksia* species in the second year (Lamont and Witkowski, 1992). Plants may continue dying over summer for at least 7 years after fire (Enright and Lamont, 1992b). Death is rare by 10 years (Lamont and Bergl, 1991).

Herbaceous weeds take advantage of bare areas for establishment. These invade stands of vegetation from roads and farms after clearing the edges or fire. At least 120 species have naturalized in the banksia woodlands (Keighery, 1989), the most aggressive of which are veld grass *(Ehrharta calycina)* and wild oats *(Avena barbarta)*. They clearly outcompete native plants for establishment especially after fire (Radford and Lamont, 1990) and increase combustibility of the ground flora. Because their progress is retarded more by spring (patchy) than autumn burns, Hobbs and Atkins (1990) have advocated spring fires in weed-infested areas dominated by Proteaceae.

6. Effects of disease

About 92% of Proteaceae assessed from southwestern Australia are considered susceptible to *Phytophthora cinnamomi* (table 2). Within some genera such as *Banksia* and *Dryandra*, all species assessed were susceptible to this pathogenic fungus. However, different species of *Hakea* and *Grevillea* ranged from resistant to highly susceptible. Variations in the susceptibility of genera are also reflected in changes in the patterns of abundance of genera.

The Proteaceae contributed most to species richness and canopy cover at most of the study sites in the Stirling Range National Park and so provide the basic structure of many plant communities in the region. Proteaceous plants had a mean projective foliage cover of 40% in healthy plant communities, but only 10% at sites with a long history of infestation by the fungus (Wills, 1993). The largest differences in abundance (as measured by cover) can be seen in banksias, which have less than one tenth the cover on sites with a long history of infestation than on comparable healthy sites. Comparison of a species list for one site at Mt. Hassell with a list compiled at the same site 14 years earlier (M. Dudzinski, pers. comm.) showed a loss of 12 species. Most notably, 10 of the 12 species that had disappeared were from the Proteaceae, and only a single plant of *Dryandra nivea* still remained (Wills, 1993). All major populations of *B. brownii* in the area are infected and the demise of this rare species appears imminent.

Table 2 . Numbers of taxa susceptible to *Phytophthora cinnamomi* and various canker fungi compared with total number assessed, and percentage difference in cover of species in 10 m x 10 m plots at healthy and old-infested dieback (> 10 years) sites, and immediate impact of canker on live cover compared with total cover (compiled by R. Wills). N.B. Cover for *Grevillea* omitted due to small sample size.

		No. Susceptible	Total No. assessed	% difference in cover	No. spp. in plots used % Δ cover
Dieback					
	All taxa	177	460	29	191
	Proteaceae	101	110	72	47
	Banksia	29	29	93	8
	Dryandra	15	15	79	10
	Grevillea	2	6		
	Hakea	16	20	52	11
	Isopogon	12	12	72	5
	Petrophile	9	9	69	6
Canker					
	All taxa	273	436	14	230
	Proteaceae	120	139	25	60
	Banksia	27	29	17	12
	Dryandra	15	17	33	9
	Grevillea	10	11	26	3
	Hakea	29	32	31	16
	Isopogon	9	10	28	4
	Petrophile	13	13	19	6

Most species of Proteaceae tend to be sensitive to aseasonal drought (Hnatiuk and Hopkins, 1980). Since species that are killed by the fungus die largely as a result of reduced ability to absorb water, it may be that species that are drought-tolerant may be more able to survive short periods of exposure to the disease, recovering when fungal activity becomes limited by temperature and/or moisture availability. It seems likely that species of *Grevillea* and *Hakea*, the only proteaceous genera that occur in arid Australia (Martin, 1982), may be more drought tolerant than other Proteaceae, and might help to explain why these are also the only genera of Proteaceae with species exhibiting lower susceptibility to *P. cinnamomi*. Currently, the most practicable management technique for the control of *P. cinnamomi* in native plant communities is the application of the fungicide phosphonate ("phosphorous acid") (Shearer and Fairman, 1991). The fungicide has a double action within plants, inhibiting fungal growth and enhancing host resistance.

In recent years, a new fungal threat has emerged. Several aerially-dispersed, canker-causing fungi have been found in a taxonomically diverse group of diseased, native plants from many plant communities in southwestern Australia and including a number of taxa classified as vulnerable or endangered. The cankers, including

Botryosphaeria spp. and *Diplodina* sp., have caused extensive damage to large stands of vegetation in south-coastal areas of Western Australia, particularly since February 1991 (Wills, 1991). It appears likely that unusual weather conditions, with 6 months of serious rainfall deficiency up until May that year, and a heat-wave lasting four days and reaching 47 °C, contributed to the rapid growth of the cankers observed in native plant communities since that time.

Studies reveal that these fungi also have a broad host range, with 62% of species assessed from a range of families affected by canker fungi (R. T. Wills and D.L. Murray, unpubl.). About 86% of Proteaceae were damaged, and often killed, by canker fungi. However, the level of damage sustained by different species was extremely variable. *Banksia coccinea* and *B. baxteri* (species restricted to south coastal areas of Western Australia) are both highly susceptible to damage by canker fungi, and as a result the commercial picking of infloresences from wild populations of these species has now been banned. While canker fungi are not a major problem elsewhere in southwestern Australia, they are distributed throughout this region and have the potential to cause very serious damage. Currently, fire is the most practicable management tool for the regeneration of native plant communities after infestation by canker.

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THE PROTEAS OF TROPICAL AFRICA

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Abstract

The genus *Protea*, while the best known as a member of the Cape Floral Kingdom with 69 species, extends over most of Africa south of the Sahara with a further 44 species. A few of these are extremely widespread, while others may be rare, localised and poorly known. Zimbabwe shares two widespread species with adjoining countries but has also five local endemic species of *Protea* as well as the only tropical species of the related *Leucospermum*. Some tropical proteas may be trees reaching 10 m in height, considered the ancestral form, while others are subshrubs whose shoots are burnt off annually in grass fires and renewed the following season. An evolutionary trend is evident from the tree to the suffrutescent form. Tropical proteas are exclusively found in highlands, some in the Brachystegia-type savanna woodlands of the great African plateaux 1 000 — 2 000 m above sea level, and others in the mountain fynbos and secondary grasslands of the mountain chains and escarpments from 1 500 to 3 000 m. While in general not as strikingly beautiful as Cape proteas, a number of the tropicals deserve introduction into horticulture.

1. Introduction

The numerous species of *Protea* which are commonly cultivated are well known to originate from the fynbos vegetation of the South African Cape Province. It may be less commonly known that the genus extends widely beyond the Cape fynbos, in fact over most of South Africa south of the Sahara. The Cape species from a tightly-knit group (Rourke, 1980) beyond which there is a group of 13 in the summer rainfall area of South Africa. Two of these are tropical species at the southern extremity of their range. North of the Limpopo a further 31 species are now recognised (Beard, 1993). The latest total therefore is 113 recognised species of *Protea* confined to Africa, of which 60% are native to the Cape Province. The other 40% are an unknown group as far as horticulture is concerned since none of them has been introduced into general cultivation and only a few experimented with in a small way. Here we have a gene pool of new material offering a challenge to growers.

2. Tropical *Protea* species

The tropical species vary from localised to extremely widespread. Some have very small ranges indeed and were for a long time known only from their type collection, while others range over almost the entire area of *Protea* country in the tropics. *P. madiensis* for example ranges from Angola across to Malawi, north into Kenya and Uganda, right up into the highlands of Ethiopia and also westward into the furthest limits of West Africa. At the opposite extreme, *P. praticola* in the highlands of southern Tanzania is so rare that only two botanists have ever seen it, and only one of those actually saw it in flower. This was W. Goetze, a German botanist who

discovered it and made the type collection in 1899. I reached the spot in 1961.

The flower-heads of tropical *Protea* spp. are for the most part not as strikingly beautiful as the Cape species. The heads are more "ordinary" with simple coloured bracts, not elongated into ornamental shapes or decked with long hairs. The most beautiful probably is *P. asymmetrica* from the Inyanga Mountains of Zimbabwe, so called because the heads do not open uniformly all round but from one side first. The tropical species do include the most bizarre of all *Protea* spp. in flower, *P. rupestris*, where the bud is enclosed in smooth reddish-purple bracts which fall away completely when the head opens, leaving the protruding flowers.

Several of the tropical species may be trees reaching 10 m, the only real trees in the genus. *P. rupestris* referred to above is a tree of this size. It ranges across Africa south of the Congo basin from Angola to Mozambique, described as a small tree attaining the canopy in stunted *Brachystegia* woodlands of especially poor soils at a high altitude (*ca.* 2 000 m, Beard, 1963). *P. petiolaris* is a smaller tree of some 5 — 6 m but with a typical tree habit, an erect trunk and ascending branches. It has much the same range as *P. rupestris*, extending also to Zimbabwe, and is named from the pseudo-petiolate leaves, so exaggeratedly narrowed at the base as to possess an apparent petiole 2 — 5 cm long. The largest tree of all are produced by a form of *P. welwitchii* which I discovered on the Morro de Moco in the mountains of western Angola and described as subsp. *mocoensis* (Beard, 1963). While it is more commonly shrubby and usually does not exceed 3 m, numerous trees of at least 10 m were observed, growing scattered in mountain grasslands associated with a new species discovered there, *P. flavopilosa* and *Faurea speciosa* (another genus of Proteaceae). *P. welwitschii* is an extremely variable species, varying in leaf size and shape, hairiness of stems and leaves, and size of flower-heads. The latter varies from large and solitary to small and clustered in groups of up to six. In consequence over 12 different species were originally described from the material, but we now take the view that only a single highly variable species is concerned (Beard, 1963, 1993).

Another type of variation in *P. welwitschii* is in growth habit. While mostly taking the form of shrubs, tree forms are found as noted above and at the other extreme miniature forms where the plants consist of numerous ephemeral stems arising from an underground rootstock. These are found in grasslands and are burnt off each year when the grass is burnt, being renewed in the spring. This adoption of both shrub and miniature forms occurs also in *P. angolensis*, another very widespread species. The two forms occur in separate habitats, the shrub form in bush and the miniature in grassland usually on the edge of *dambos* (treeless, swampy valley bottoms), but they are about equally widespread. In this case the two forms being quite distinct are recognised as varieties, var. *angolensis* for the miniature and var. *divaricata* for the shrub form.

Other tropical species are less variable in this respect and tend to be consistently either shrubs or miniature ("suffrutescent"). Of the 33 tropical species, 15 are consistently suffrutescent and may be considered as having evolved to suit the fire-prone habitat of the tropical savanna. The tree habit is considered ancestral, evolving to the shrub habit and this in turn to the suffrutescent form (Beard, 1993).

Colour of flower-heads is also variable, from red through pink to white. Many Cape species have both pink and white heads mixed in the same population, but this is not so marked in the tropical group. Only in *P. angolensis* perhaps is it a

noteworthy feature, where both the miniature and shrubby varieties are generally white but the miniature has a pink form found only in N.E. Zambia (Chisumpa and Brummitt, 1987) and the shrubby variety a brilliant red form rather more widespread in Zambia and Malawi. In general, bracts and flowers are pink-tinged in tropical species.

Broadly, the tropical *Protea* species occupy two principal habitats, the one related to that of Cape species, the other quite distinct from it. The former is vegetated as in the Cape by fynbos or by degraded communities derived from it, the latter by plateau woodland, that vast complex of savanna woodlands of the *Brachystegia-Isoberlinia-Julbernardia* assemblage which covers so much of Africa south of the Sahara. The Cape fynbos is generally classified, like Western Australia kwongan (Pate and Beard, 1984), as 'Mediterranean-type' vegetation supposedly adapted to a wet winter, dry summer climatic regime. The latter concept is debatable as the component plants do not exhibit any specific adaptations of the required nature and in both continents, as well as in California, this vegetation extends outside the area of strictly mediterranean climate. The fynbos extends from the winter rainfall western Cape into the constant rainfall zone and then on beyond Grahamstown into the summer rainfall zone. Here its presence is less obvious because summer rainfall promotes the growth of grass, which becomes dry and inflammable in winter, with the result that fire has largely eliminated the fire-tender fynbos in favour of grassland. Sufficient relics of it survive to attest its former presence and composition, and some fire-resistant members of it persist, forming a shrub-savanna vegetation in combination with the grassland. These often happen to be proteas and a protea savanna derived in this way is a frequent element together with fynbos relics all along the escarpments and mountains of eastern Africa at ever increasing altitude until it becomes the "ericaceous zone" of East African mountain at an altitude of some 3 000 m (Hedberg, 1951).

The great plateaux of tropical Africa extends between altitudes of 1 000 and 2 000 m and is vegetated with a grassy woodland of leguminous trees. Famous experiments in Zambia (Trapnell, 1959; Werger and Coetzee, 1978) have shown that the true climax here is a semi-evergreen forest ("muhulu") which will develop under fire protection. The *Brachystegia* woodland ("miombo") is a secondary formation maintained by early, light burning. Late season, severe burning creates open grassland. *Protea* spp. are inhabitants of the miombo at the higher elevations and on the poorer and rockier soils where the trees are stunted and less competitive with the smaller *Protea* spp. This is where the tree proteas are found with other species which possess characters considered primitive in the evolutionary sense. It is here that the ancestral home of African proteas appears to lie.

In Australia the shrubby, sclerophyllous Proteaceae of kwongan and other "heathlands" are believed to be descended from rain-forest ancestors, and proteaceous genera are still represented among rain forest trees. This is also the case in Madagascar where a single monotypic genus *Dilobeia* occurs. This taxon does not occur in Africa (Johnson and Briggs, 1975). The family is not represented, today at least, in African rain forests. It is thought that *Faurea*, another proteaceous genus represented in both tropical Africa and Madagascar, is possibly ancestral (Rourke, 1972). This is primarily a tree of closed and open hill forests in the drier parts of the tropics, not of rain forest. The only other genus of Proteaceae represented in the

African tropics is *Leucospermum* with a single species *L. saxosum* in the Chimanimani mountains of Zimbabwe.

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COMMERCIAL PICKING OF *BANKSIA HOOKERIANA* FROM NATURAL POPULATIONS ADVERSELY AFFECTS SHOOT, FLOWER AND SEED PRODUCTION AND PLANT NUTRIENT STATUS

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Abstract

The impact of bloom picking on subsequent flower and cone production, seed bank dynamics and plant architecture were studied in three picked and three unpicked populations of *Banksia hookeriana* near Eneabba, Western Australia. Losses of dry mass and mineral nutrients, via commercial bloom picking and flower head removal by cockatoos were quantified. A total of 13 255 blooms were picked ha^{-1} over the last 9 years, or 29% of production. Cockatoos removed a further 2 477 flower heads.ha^{-1} (5%). Picking reduced plant canopy volume by 44% and the number of 1 year old stems by 56%. Picked plants produced 35% fewer blooms, while seed production and storage were reduced by 50% and 57% respectively. Aerial dry mass and N and P contents were reduced by 20.3%, 29.8% and 31.3%, respectively, whereas cockatoos removed only 1.1%, 1.3% and 1.7% during the same period. Ecosystem losses of N and P at 3103 g $N.ha^{-1}$ and 152 g $P.ha^{-1}$ since the last fire were substantial, but losses from cockatoo damage were virtually negligible. The reduced seed store with picking may adversely affect post-fire regeneration, especially in the event of a short fire interval. Sink (inflorescence) removal reduced the nutrient status of the plant, especially in terms of N, due largely to the loss of nutrients and energy stored in the recently mature leaves supported on the picked stem.

1. Introduction

Commercial flower picking in Australian heathlands has escalated in recent years, resulting in concern for the long-term maintenance of favoured species in the wild. Many of these have restricted distributions and face a myriad number of threats, including clearing for mining and agriculture, and exotic diseases. *Banksia hookeriana* Meissner is a sclerophyllous fire-killed shrub with cone-like flower heads highly favoured for the export wildflower trade (Witkowski *et al.*, 1991). It is restricted geographically to an area of 33 x 74 km in the northern sandplains of Western Australia centred on Eneabba, and topographically to the crests and upper slopes of sand dunes (Lamont *et al.*, 1989). Little information is available on the extent of flower-picking in the wild, or its effects on population maintenance and both plant and ecosystem nutrient status. *B. hookeriana* is dependent on canopy-stored seed after fire for regeneration, and seedling recruitment is restricted to the first winter/spring period after fire. Reduction in the potential seed store in response to intensive harvesting could result in insufficient recruitment for the replacement of parent plants. In addition, cockatoos also damage flower heads and cones of many species of *Banksia* while feeding

on phytophagous insects.

These dunal sands have very low mineral nutrient contents (Witkowski *et al.*, 1993a), yet the seeds of banksias have very high nutrient contents. Thus nutrient supply may be a major limiting factor in seed and fruit production. Bloom harvesting removes nutrients, but it also leaves fewer sinks for nutrients, so that seed set in the remaining cones might increase.

2. Materials and Methods

Plant size, the number of blooms produced, seed bank dynamics, plant architecture and nutrient losses through picking and cockatoo damage were compared in three intensively picked and three negligibly picked (unpicked), but otherwise closely matched, populations of *B. hookeriana*. All populations were 13 years old and occurred on the mid-slope position on the dunes.

3. Results

3.1. Plant density and dimensions

Densities of *B. hookeriana* and all other shrubs were significantly higher in the unpicked sites (table 1). This may be a response to bloom harvesting in the previous cohort of *B. hookeriana* (i.e. before the last fire). The unpicked plants were taller, with much larger canopy areas and volumes (table 1).

3.2. Bloom harvesting impacts

Commercial pickers removed 29.4% of the blooms produced over the last 9 years, since these plants became reproductively mature at 4 years of age. Only 1.8% were removed from the "unpicked" plants. Cockatoos removed a further 5.3% and 7.3% of flower heads respectively. This harvesting pressure resulted in a significant reduction in bloom production per plant (35%; table 1). On an area basis, this amounted to an even bigger reduction in bloom production of 55%. The actual average reduction in production was from 100 000 blooms.ha^{-1} in the unpicked sites to only 45 000 blooms.ha^{-1} in the picked sites.

3.3. Seed bank dynamics

The number of fertile cones remaining on the picked plants after the combined effects of commercial picking and cockatoos was only 46.5% of that in the unpicked plants (table 2). However, the proportion of cones that were fertile was not affected. Seed production per follicle and the number of follicles per fertile cone were also unaffected by picking. Nevertheless, total seed production per plant was reduced by 50%, and this reduction may be almost totally attributed to the reduced number of fertile cones on these plants.

As the cones aged from 1 to 8 years, the proportion of seeds dispersed (released) increased from negligible numbers to 32-54% (figure 1). The percentage of seeds eaten increased from about 10% to 20% in the old cones, while seed abortion was unaffected

by cone age. The percentage of viable seeds decreased from about 60-80% to only 10 — 20% in the old cones (figure 1). Thus the newer cones are a much more important source of viable seeds than the older ones. Time to first germination and to 50% germination increased progressively with cone age (figure 2). These trends were similar for both the picked and unpicked plants. There were small differences in the overall fate of these seeds between treatments: namely, there was a small increase in the number of seeds eaten by granivores, and slightly more tended to be released and non-viable in the picked relative to the unpicked plants (table 2). As a result, the actual viable seed store was reduced by an even greater amount, 57%. The average reduction per plant was from 848 seeds in the unpicked to 361 seeds in the picked plants (table 2). The number of viable seeds stored in the canopy is a negative exponential function of bloom harvesting (figure 3). The impacts of this depleted seed store on post-fire seedling recruitment is uncertain. However, applying a lottery population model, which assumes equal probability of any seed of any species occupying one of a finite number of suitable microsites for establishment after fire, successful establishment of *B. hookeriana* seedlings will be substantially reduced. Indeed, because other species at these sites are likely to have increased their seed production in response to reduced competition from picked *B. hookeriana*, the overall reduction in *B. hookeriana* seed numbers relative to other species will be greater still. A similar lottery model has been produced for *B. attenuata* (Enright and Lamont, 1992).

3.4. Plant architecture

There was a 44% reduction in total annual stem increments per plant and a 56% reduction in 1 year old stems (table 2). The number of year 1 branches per apex was also significantly reduced. Commercial picking opened up the canopy, allowing 3x more light to pass through to the cones held within it. The greater exposure to radiation is likely to have been the cause of the increased percentage seed release and predation (table 2). Very few stems resprouted after commercial bloom removal, but a much higher proportion did resprout after cockatoo flower head removal (table 2). Commercial bloom picking involves the removal of the flower head, a 300 mm long supporting stem (representing 2.2 annual growth increments) and the recently mature leaves held on the stem. These parts contain considerable amounts of nutrients and energy, as well as viable axillary buds. Because cockatoos only remove the flower head, most of the growing points are retained and energy and nutrient reserves are depleted to a much lesser extent. This would explain the greater resprouting vigour in cockatoo damaged relative to commercially picked stems.

3.5. Plant and ecosystem nutrient status

Above-ground dry mass and N and P contents per plant were reduced by 20.3%, 28.6% and 29.6% respectively from commercial offtake, whereas cockatoos removed only 1.1%, 1.3% and 1.7%, respectively (Witkowski *et al.*, 1993b). Removal of K, Ca, Mg and Na were in the same order of magnitude. On an area basis, losses of N and P were 3 103 g $N.ha^{-1}$ and 152 g $P.ha^{-1}$ since the last fire. The removal of 240 g $N.ha^{-1}$ and 14 g $P.ha^{-1}$ by cockatoos was not a "loss" to the ecosystem as the birds dropped the flower heads near the plants. Concentrations of N in the blooms of the

picked plants were reduced by 15% for flower heads, 32% for leaves and 65% for stems relative to the unpicked plants. In contrast, P concentrations were 22% higher in the leaves and stems of the picked blooms. In terms of nutrient contents, N was 22% lower overall in the blooms of the picked plants, while P contents were not significantly different between treatments, but tended to be higher in the picked blooms (11%). About half of above-ground P (50.4%) and a quarter of N (24.4%) were allocated to seeds, and thus seed production is a very large drain on plant nutrients. Considering losses from cone removal (as done by commercial seed collectors), it is clear that very high fractions of above-ground P and N would be lost if cones were harvested, instead of blooms, at the same rate of 29.4% of overall production.

Table 1. Densities and plant dimensions (means ± S.E.), total flower head production and removal by pickers and cockatoos of *Banksia hookeriana* from picked and unpicked populations near Eneabba, Western Australia. Nested ANOVA of: (a) 3, 20 x 10 m quadrats for density data, (b) 20 plants for dimensions, and c) 10 plants for all other data, from each of 3 populations per treatment. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

Attribute	Picked	Unpicked	Prob.
Plant density (plants ha^{-1})			
B. hookeriana	867 ± 237	1256 ± 311	*
Other shrubs	8356 ± 1557	10389 ± 2384	**
Plant Dimensions			
Height (m)	1.55 ± 0.03	1.71 ± 0.05	***
Canopy area (m^2)	2.65 ± 0.22	4.21 ± 0.42	***
Canopy volume (m^3)	2.83 ± 0.28	5.05 ± 0.63	***
Flower heads per plant			
Total produced	53.7 ± 3.9	82.7 ± 7.0	***
Removed by pickers	15.7 ± 1.7	1.4 ± 0.3	***
Removed by cockatoos	2.8 ± 0.4	6.4 ± 1.3	***
Total removed	18.5 ± 10.6	7.8 ± 7.3	***
Total flower heads per hectare			
Produced	45216 ± 3611	100328 ± 9940	***
Removed by pickers	13255 ± 1537	1787 ± 448	***
Removed by cockato	2477 ± 371	7562 ± 1384	***
Total removed	15733 ± 9834	9350 ± 8464	***

4. Conclusions

Overall levels of bloom picking in these three wild populations over the last nine years (and 13 years since the last fire) was 29.4% of production, a substantial proportion. The indirect effects of picking were also substantial as harvesting resulted

in a reduction in the canopy-stored seed bank of 57% relative to the negligibly picked plants. Contrary to expectations, bloom removal did not stimulate compensatory stem and bloom production, but had the reverse effect. Indeed cone production was reduced by 35% and the number of 1 year old stem apices by 56%. Furthermore, seed set per cone did not increase in the remaining cones and did not differ between treatments. By greatly reducing the number of seeds stored, bloom picking may adversely impact on post-fire seedling recruitment, which could lead to the decline of an already restricted species. Commercial bloom picking not only removed potential nutrient sinks (the developing fruits), but also a large proportion of the most recent and metabolically active leaves. This ultimately appeared to adversely affect the nutrient status of the plant, particularly in terms of N, reducing subsequent growth, nutrient uptake, and bloom production.

Table 2. Comparison of overall seed bank dynamics and plant architectural traits (means ± S.E.) between picked and unpicked populations of *Banksia hookeriana* near Eneabba, Western Australia. Nested ANOVA of (a) 10 plants for cones, follicles and seeds, (b) 20 plants for canopy openness, and (c) 5 plants for the other comparisons, from each of 3 populations per treatment. **, $P < 0.01$; ***, $P < 0.001$; NS, not significant ($P > 0.05$).

Attribute	Picked	Unpicked	Prob.
Cones			
Fertile cones per plant	31.8 ± 2.8	68.4 ± 5.5	***
Cone fertility (%)	90.5 ± 1.8	92.1 ± 1.2	NS
Follicles per fertile cone	11.4 ± 0.5	10.1 ± 0.4	NS
Seed production			
Seeds per follicle	1.7 ± 0.02	1.7 ± 0.02	NS
Percentage zygotes	83.7 ± 0.9	85.1 ± 1.1	NS
Production per plant	563 ± 60	1125 ± 93	***
Seed fate per plant			
Released (%)	8.5 ± 1.9	5.6 ± 0.5	NS
Eaten (%)	16.6 ± 1.1	12.8 ± 0.6	***
Aborted (%)	16.3 ± 0.8	14.9 ± 1.0	NS
Non-viable (%)	3.8 ± 0.5	3.2 ± 0.4	NS
Viable (%)	54.8 ± 1.7	63.5 ± 1.0	***
Viable seed store per plant	361 ± 38	848 ± 75	***
Plant architecture			
Total annual stems per plant	257 ± 21	471 ± 41	**
1-year-old stems	56 ± 7	128 ± 19	**
Stems removed (%)	15.4 ± 2.1	1.0 ± 0.4	***
No. of year 1 branches per apex		1.22 ± 0.05	1.35 ± 0.03NS
Canopy openness (%)	24.7 ± 2.2	9.3 ± 0.8	***
Stem resprouting			
From picking (%)	24.2 ± 4.1	0.0 ± 0.0	–
From cockatoo damaged (%)		63.5 ± 8.6	52.9 ± 5.4NS

5. Management recommendations

There are several management implications arising from these results. We propose that no picking be allowed for the first 8 years after fire to allow these shrubs to produce a small seed bank, thus allowing some regeneration in the event of an unusually short fire interval. In addition, harvesting should be reduced to 20% of the blooms produced per year to ensure that the seed bank remains above 50% of that of the unpicked populations. We believe that these measures will actually result in potentially the same numbers, or even more, blooms per hectare available for harvesting. This is because these larger more vigorous plants will have much higher productive abilities than those that are currently harvested at a much younger age and at a higher intensity. Only the highest quality blooms should be picked so that fewer blooms have to be discarded on quality grounds after harvesting. Although long stems are desirable, any increase beyond the 300 mm norm currently employed for wild-flower harvesting of *B. hookeriana* will probably have an additional adverse impact on plant vigour. The picking of fertile cones for their seeds should be strictly controlled to safeguard against ecosystem nutrient depletion and loss of stored seed. Where fertile cone harvesting is allowed, it should be only on relatively old plants (> 12 years), it should be evenly spread throughout the area and not localized (to prevent local extinction), and no more than 20% of fertile cones per plant should be removed. If leaves were stripped from the bloom at the point of harvesting, ecosystem nutrient losses would be reduced by 35-57%. Control burns in picked areas should be no more frequent than every 15 years, i.e. 5 years longer than the minimum recommended for unpicked scrub-heath in this region (Cowling and Lamont, 1987). The levels of bloom harvesting of *B. hookeriana* and other restricted species in the wild needs to be closely monitored to prevent population decline and to ensure their survival in the long term. Finally, local farmers should be encouraged to grow *B. hookeriana* commercially for bloom production on previously cleared land to alleviate pressure on the wild populations.

6. Research recommendations

The effects of reduced seed storage in response to bloom harvesting on post-fire seedling recruitment under various fire seasons and frequencies is a high priority for further research. Farmers encouraged to grow *B. hookeriana* and other local species on their previously-cleared land, should have the support of ongoing horticultural research to help improve both yields and quality. The results on ecosystem nutrient depletion in this study are highly indicative that depletion is occurring. However, long-term experimental studies appear to be the only means to detect unambiguously nutrient losses from the soil . Furthermore, we also need to know to what extent nutrient removal from these ecosystems is sustainable without causing reductions in population sizes and species survival.

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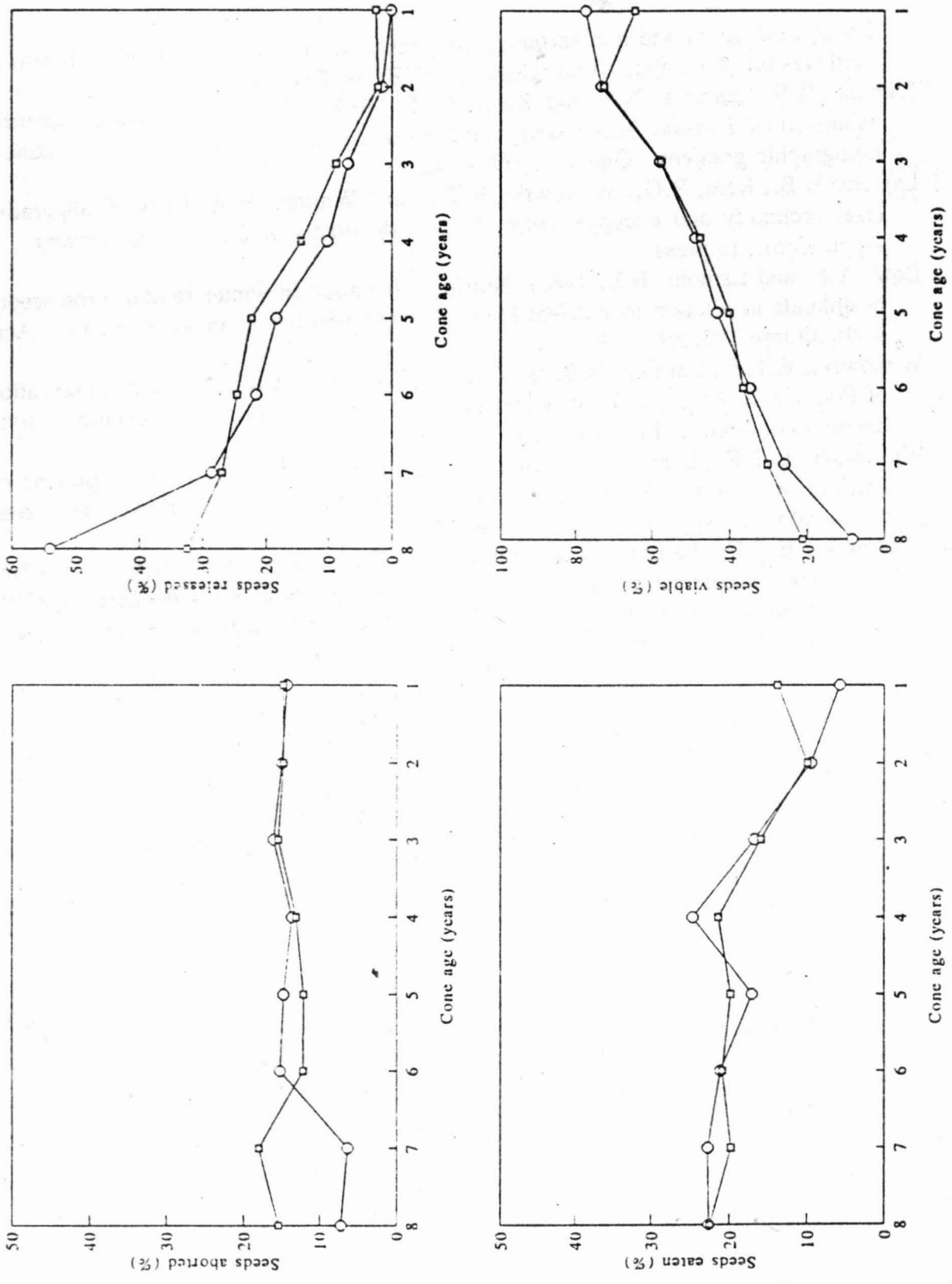

Figure 1. Percentage of seeds aborted, released, eaten and viable (stored in the canopy) of picked (□) and unpicked (●) *Banksia hookeriana* shrubs in relation to cone age.

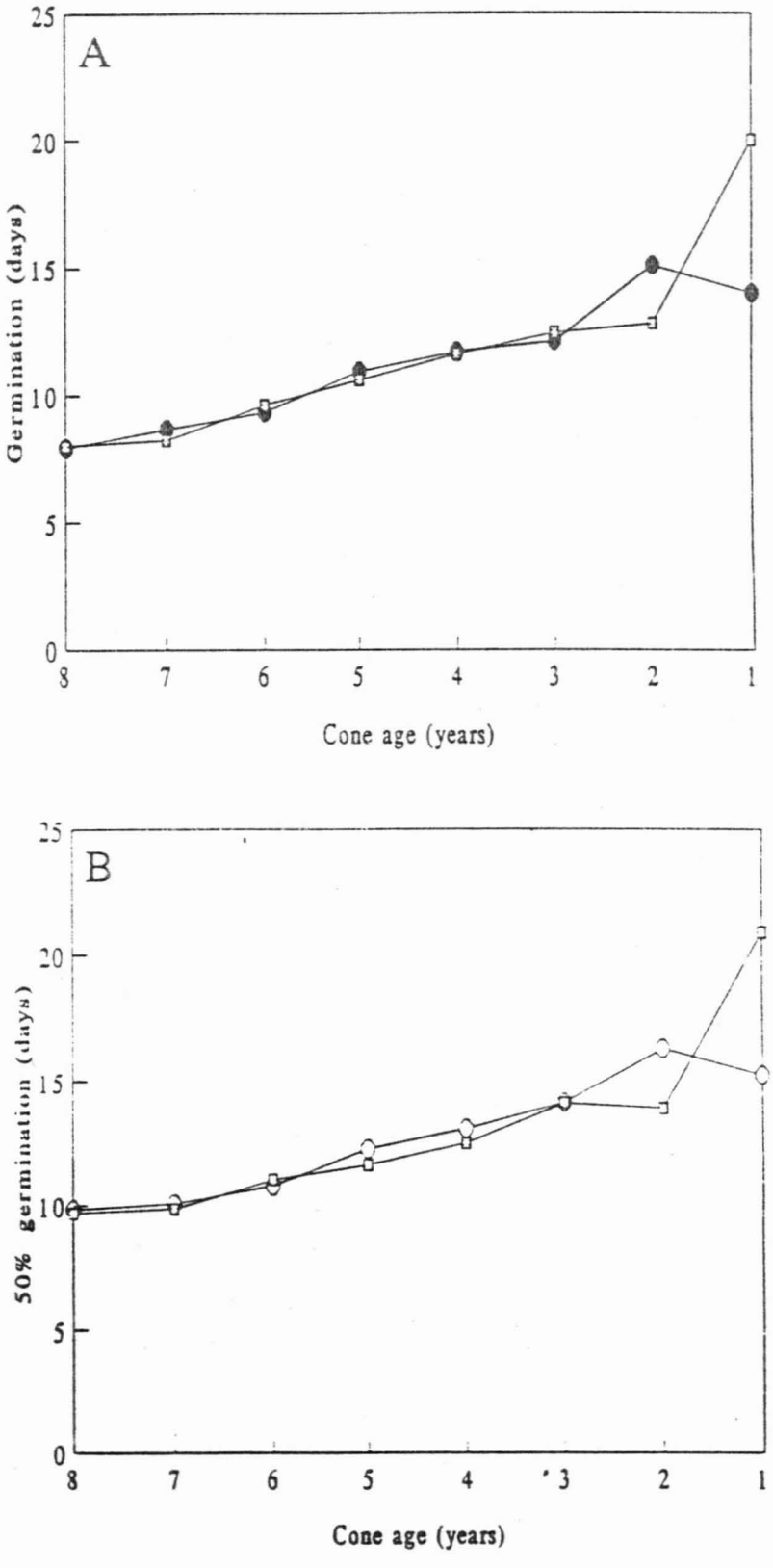

Figure 2. (A) Time to first germination and (B) time to 50% germination of seeds of *Banksia hookeriana* in relation to cone age (= seed age) in picked (□) and unpicked (O) populations.

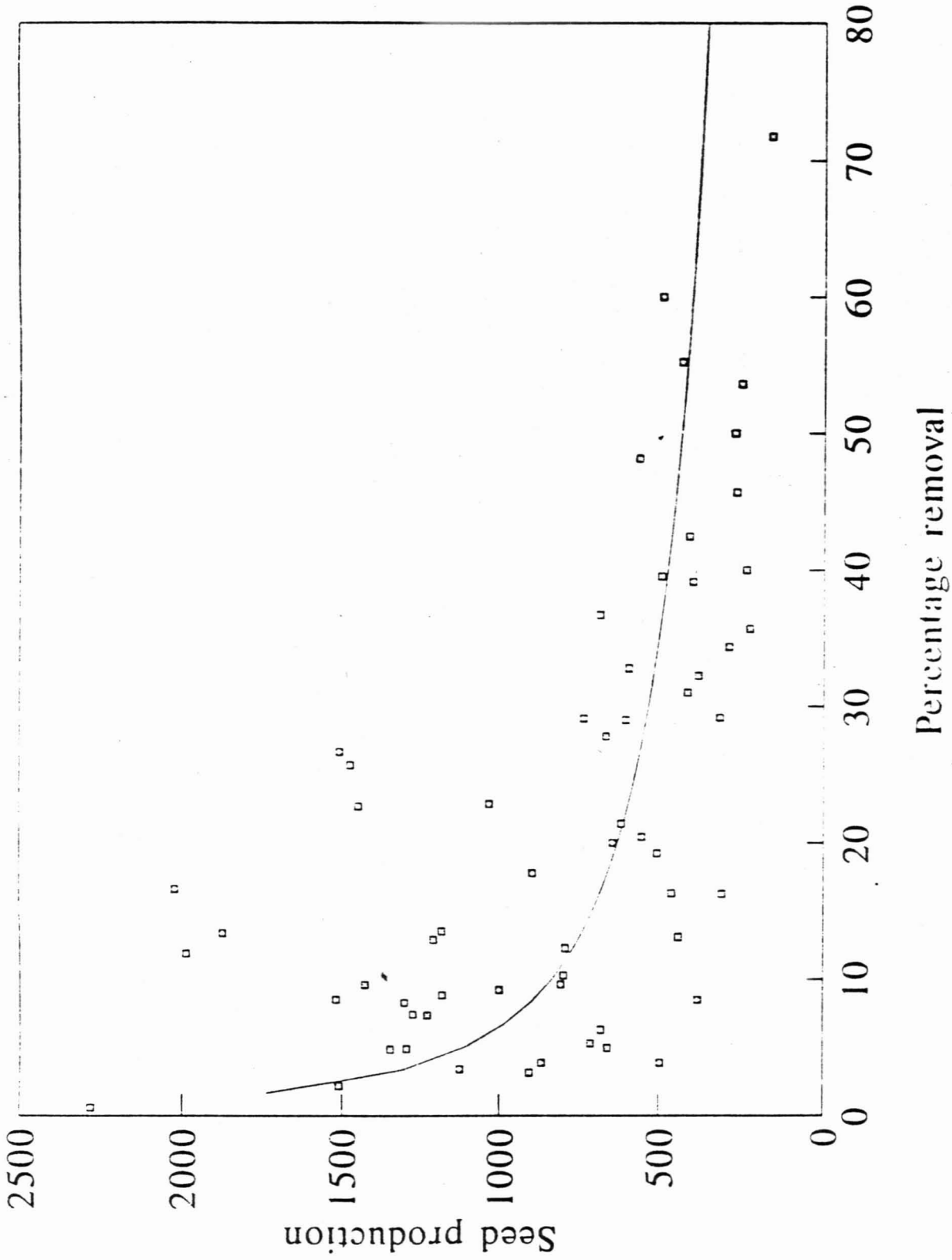

Figure 3. Life-time seed production of 13-year-old *Banksia hookeriana* shrubs in relation to the percentage of cones removed (combined effects of human pickers and cockatoos).

THE DEVELOPMENT OF *LEUCOSPERMUM* AND *SERRURIA* AS FLOWERING POT PLANTS

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Abstract

Attractive and unique pot plants can be produced from some of the South African Proteaceae.

In a research project carried out in South Africa, the growth and flowering of *Leucospermum* and *Serruria* pot plant cultivars and selections were evaluated during production and for their post-harvest performance.

Four production systems were evaluated, which were based on different types of cuttings:

1. Non-induced, unbranched hardwood cuttings rooted in spring. These cuttings were branched in pots during production. They flowered after 12 months.
2. Non-induced, branched softwood cuttings rooted in late summer. These cuttings flowered after 7 — 8 months.
3. Induced, branched semi-hardwood cuttings, rooted in late autumn. These cuttings flowered after 6 months.
4. Eighteen-month to two-year production. Branched cuttings rooted as in systems 2 or 3 but kept under production for an additional year to evaluate their performance in the second flowering season.

Leucospermum and *Serruria* selections were tested under the several production systems by evaluating their rooting ability and the time required to produce flowering pot plants.

Three large, single-flower cultivars of *Leucospermum* were evaluated. 'Ballerina' was the best because 90 — 100% of the plants produced acceptable flowering pot plants, depending on the production system. Next were 'Tango' 42 — 100% and *L. tottum* producing 10 — 100% acceptable flowering pot plants.

With the small multi-headed species *L. mundii* and *L. oleifolium*, acceptable flowering pot plants were produced only after 17 months of cultivation.

Interspecies, F1 hybrids of *Serruria florida* x *S. rosea* rooted well, above 88%, in January-February and 20-85% of the plants produced acceptable flowering pot plants within 6 months of propagation.

Contribution from the Agricultural Research Organization, The Volcani Center, Be Dagan, Israel. No. 1241-E, 1993 series.

1. Introduction

Proteas have become world famous for their magnificent flowers and are popular as cut flowers and garden plants. Recently, they have also been tried as a new product: pot plants.

In Europe and in other countries, the market for flowering potted plants is expanding at the expense of traditional cut flowers. The flowering pot plant industry is modern and efficient, and it constantly demands new types of product. There is no doubt that attractive and unusual pot plants can be produced from some of the South African Proteaceae. These flowering plants can be an important addition to the pot plant industry.

Leucospermum and *Serruria* have potential as flowering pot plants for export, provided they can be produced with acceptable quality and supplied at a reasonable price. In order to stay competitive it is important to produce flowering protea pot plants in a year or less (Ackerman *et al.*, 1992; Ben-Jaacov *et al.*, 1986; Brits, 1989b; Brits *et al.*, 1992).

Woody species such as proteas are difficult to develop as pot plants and, therefore, are still not common on the market. They are difficult to produce because they are slow to root, they do not flower readily at a young stage and they require high light intensities to flower (Brits, 1989a).

The aim of the present study was to evaluate several production systems which could possibly shorten the production time of flowering pot plants, using some of the new cultivars developed in South Africa.

2. Materials and Methods

2.1. Plant material

Four-year-old *Leucospermum* and *Serruria* stock plants were grown at the Tygerhoek and Elsenburg plantations in the Republic of South Africa. Three *Leucospermum* cultivars with large (7 — 10 cm diameter) flower heads were used: 'Ballerina' (*L. lineare* x *L. tottum*), 'Tango' (*L. lineare* x *L. glabrum*), and clonal selections of *L. tottum*. In addition, two small (2 — 4 cm diameter) multiple-head selections of each of *L. mundii* and *L. oleifolium* were used. Furthermore, we tested several cultivars and clones of *Serruria* F_1 hybrids of *Serruria florida* x *S. rosea:* 'Bridesmaid', 'Fairy', T89 08 11, T89 08 14, T90 09 12, PLT-6.

2.2. Preparation of cuttings and production systems

Four production systems were evaluated for flowering pot plants, based on the propagation of different types of cuttings:

System 1 — Non-induced, unbranched, hardwood cuttings rooted in spring.

These cuttings were branched in pots during production. After flowering was completed in the spring, and before new shoots sprouted, hardwood cuttings were harvested and rooted. After rooting and potting, the single shoot cuttings were sprouted

and branched naturally in the pots.

System 2 — Non-induced, branched, softwood cuttings rooted in late summer.

These cuttings were branched on the mother plants. Mother plants were pruned in the first week of September, October or November (spring) 1990. Strong elongating shoots were headed back to a length of 5 — 10 cm. Four weeks after pruning, the newly sprouting lateral shoots were thinned out, leaving the three strongest shoots on each bearer. When these shoots reached an average length of 10 cm they were induced to branch by spraying them with ethephon at 960 mg/ℓ. The branched, non-induced cuttings were rooted during March (late summer).

System 3 — Induced, branched, semi-hardwood cuttings, rooted in late autumn ('rapid production system').

These cuttings were branched on the mother plants. These branched, induced cuttings were prepared as described for system 2, but rooted during May (late autumn). Inflorescence buds on these cuttings had already developed up to the floret initiation stage (Brits, 1989b; Brits *et al.*, 1992).

System 4 — Eighteen-month to two-year production.

Branched cuttings were rooted as in systems 2 or 3 but were kept under production for an additional year, to evaluate their performance in the second flowering season. This system was used only with cultivars that did not flower satisfactorily in the other systems mentioned above (i.e. less than one year).

2.3. Rooting

At least 25 cuttings (see table 1), 15 — 25 cm long were used for each treatment (system x cultivar). The cuttings were dipped for 10 sec. in an aqueous solution of K-IBA (4 000 ppm) and each was stuck in a perforated plastic bag (10 cm deep x 7 cm diameter) containing rooting medium of peat, sand and polystyrene (1:1:1). The plastic bags were placed in propagating trays (plastic or wooden boxes 29 x 30 x 15 cm) with drainage holes. The propagating trays were placed in beds and misted automatically as needed.

Temperatures at the base of the cuttings were maintained at 22 °C for spring and late-summer rooting (systems 1 and 2). The temperature was uncontrolled (8 —18 °C) for fall rooting (system 3).

2.4. Growth conditions

Rooted cuttings were potted in plastic pots (12 cm diameter) containing peat, pine bark and river sand (10:40:50). The potted plants were acclimatized for 1 month under 40% shade cloth and then transferred outdoors and placed on black plastic sheeting. The plants received overhead irrigation by microjet (32 ℓ/h) for 15 min., twice daily in the summer and twice weekly in the winter. Every irrigation included

fertilization (Ackerman and Brits, 1991). A final solution of the following elements, in ppm as enumerated was applied via venturi equipment: 77 N; 63 K; 5 P; 8 Mg; 23 Ca; 1.8 Fe and microelement complex ('Korateen').

2.5. Cold storage treatment and shelf life

Six to ten uniform plants, of each of the cultivars being tested — *Leucospermum* 'Ballerina', *L. oleifolium, L. mundii* and *Serruria* cultivars 'Bridesmaid' and 'Fairy' — were used for the post-harvest studies.

Preliminary experiments showed that 4°C and 90% RH were the best storage conditions; these conditions were used in all subsequent experiments.

Shipments were simulated by placing the plants in cartons with ventilation holes, for 4 or 8 days, in darkness. After this simulated shipment, the plants were transferred to the observation room, which simulated an indoor environment: light intensity of 15 $\mu mol.s^{-1}.m^{-2}$, was provided by fluorescent tubes for 12 h daily. Temperature was maintained at 20 °C and RH at 60% (day and night).

2.6. Stage of inflorescence opening

Proteas are sun-loving plants and do not flower under low light intensities. Preliminary experiments showed that it is very important to bring flowering pot plants indoors only after the flower buds reach a certain minimum stage of development. Young flowering buds would fail to continue their development and would abort under low indoor light, whereas more developed flower buds continue their development even under low light. These minimum stages are: for *Leucospermum*, when the first row of styles is released on the inflorescence, and for *Serruria*, when some bracts start to separate from the cone-shaped inflorescence bud.

The duration of shelf life was determined visually. Plants were discarded when they reached the stage of being unacceptable as decorative objects, i.e., when the flower heads started wilting, turned black or dropped, and the leaves lost their green colour.

3. Results

Leucospermum:

3.1. Rooting ability of *Leucospermum* cuttings

Differences were found in rooting ability of the several types of cuttings, according to the seasons and the selections used (table 1). When non-induced, unbranched hardwood cuttings of different species and cultivars were stuck in the spring, very high (84 — 97%) rooting percentages were obtained within 10 weeks. In *L. oleifolium* T 85 09 05 the rooting percentage was only 76% (table 1a).

When non-induced, branched, softwood cuttings were stuck in summer, high rooting percentages of 'Tango' and 'Ballerina' were obtained after only 4 — 5 weeks, whereas lower rooting percentages were obtained for *L. mundii* and *L. oleifolium* selections (table 1b).

Induced-branched, semi-hardwood cuttings of 'Tango', 'Ballerina' and *L. tottum*

stuck in autumn resulted in relatively low rooting percentages. Very low rooting was obtained for *L. mundii*, and *L. oleifolium* (table 1c).

3.2. Growth and flowering of *Leucospermum* pot plants under outdoor conditions

Table 2 shows the growth performance and flowering ability of the various cultivars and species under the four production systems. When the flowering abilities of the three large-head varieties in the four production systems were compared, it was found that 'Ballerina' was superior because of its ability to produce 90 — 100% flowering plants in all production systems. The high flowering rates of 'Tango' and *L. tottum* were obtained when production systems 2 and 4 were used (86 — 100%). The reason for the low percentage of flowering plants obtained in system no. 3 was the abortion of flower buds during rooting.

In *L. tottum*, secondary shoot diameter was markedly influenced by production system. The low percentage obtained when production systems 1 and 3 were used was due to the high percentage of thin branches (< 2,5 mm in system 1) and abortion of flower buds during rooting (in system 3).

Selection T 85 09 04 of *L. oleifolium*, using production system no. 4, produced an average of eight inflorescences per plant, and 100% of the plants flowered after 17 months.

Under the other production systems, low percentages of the plants flowered. Under production system 4, *L. mundii* produced 12 inflorescences per plant and only 83% of the plants flowered after 19 months. The use of other production systems was less effective.

3.3. Shelf life of *Leucospermum* pot plants after simulated shipment

It was found that cold storage of 'Ballerina' pot plants in darkness for 4 or 8 days did not cause any damage or reduction in quality. 'Ballerina' plants continued flowering under home conditions for 3 weeks, producing high-quality, long-lasting flowering pot plants (table 6).

Leucospermum oleifolium continued flowering for 17 days, but suffered from blackening of buds and discoloration of some of the leaves. Plants of *L. mundii* flowered for 7 — 10 days, with slight discoloration of the leaves.

Serruria:

3.4. Preparation of branched *Serruria* cuttings on mother plants

Preparation of branched cuttings and rooting procedures for *Serruria* were similar to those for *Leucospermum* (see Material and Methods). The attempt to induce secondary branching by ethephon spray (during November) failed to produce branching (ethephon was probably applied at too late a stage of shoot growth — unpublished results).

Since no branched cuttings of *Serruria* were available, single 14 cm non-induced hardwood, non-induced softwood and induced semi-hardwood cuttings were used (parallel to systems 1, 2 and 3, except that all cuttings were unbranched).

3.5. Rooting ability of *Serruria*

Rooting percentages of unbranched cuttings of *Serruria* selections are presented in table 3. Non-induced hardwood cuttings (system 1) of *Serruria*, rooted in spring (6/9/91) resulted in low rooting percentages. Non-induced cuttings (system 2) of all selections stuck in summer (January — February), showed high percentages of rooting (88 — 100%). Induced semi-hardwood cuttings (system 3) stuck in late autumn (25/5/91) resulted in low rooting percentages.

3.6. Flowering of *Serruria* under outdoor conditions

Dates of flowering, the length of the flowering period and flowering percentages of *Serruria* pot plants selections under outdoor conditions are presented in table 4. All selections developed an average of 1 — 3.4 inflorescences per cutting (plant). The largest and most attractive flowering pot plants produced were of 'Bridesmaid' and 'Fairy'.

Serruria 'Bridesmaid' started flowering on 22/04/91, four weeks earlier than any other cultivars tested (these started flowering from mid-May to mid-June). The flowering seasons of 'Bridesmaid' and 'Fairy' lasted 105 and 81 days respectively, whilst other selections had shorter flowering periods.

3.7. Shelf life of *Serruria* flowering pot plants

Preliminary experiments, using simulated shipping conditions showed that 4 °C and 90% RH were the best storage conditions. These conditions were used for 4 or 8 days in all subsequent experiments.

Flowering pot plants of the above-mentioned best *Serruria* cultivars, 'Bridesmaid' and 'Fairy', were at different stages of inflorescence development when they were placed in the observation room.

Table 5 shows that inflorescences from these two cultivars had a life span of 30-55 days and that they produced very satisfactory, long-lasting flowering pot plants.

4. Discussion and conclusions

In this study, the propagation, cultivation and shelf life of flowering pot plants of *Leucospermum* and *Serruria* were investigated.

4.1. *Leucospermum*

Flowering pot plants of *Leucospermum* species and cultivars could be divided into two groups, differing in vegetative and flowering habits.

Group A — Single large flower heads: This group is characterized by large, single, terminal inflorescences (diameter 7 — 10 cm — figure 1). It includes *L. tottum*, 'Tango', 'Ballerina'. This group is characterized by quick rooting and high rooting percentages and good growth rate; they are also able to flower on short thin shoots, produced after the branching treatments.

Table 2 shows that every branch produced an average of one terminal inflorescence (figure 1). An additional 2 — 3 lateral inflorescence buds may be produced

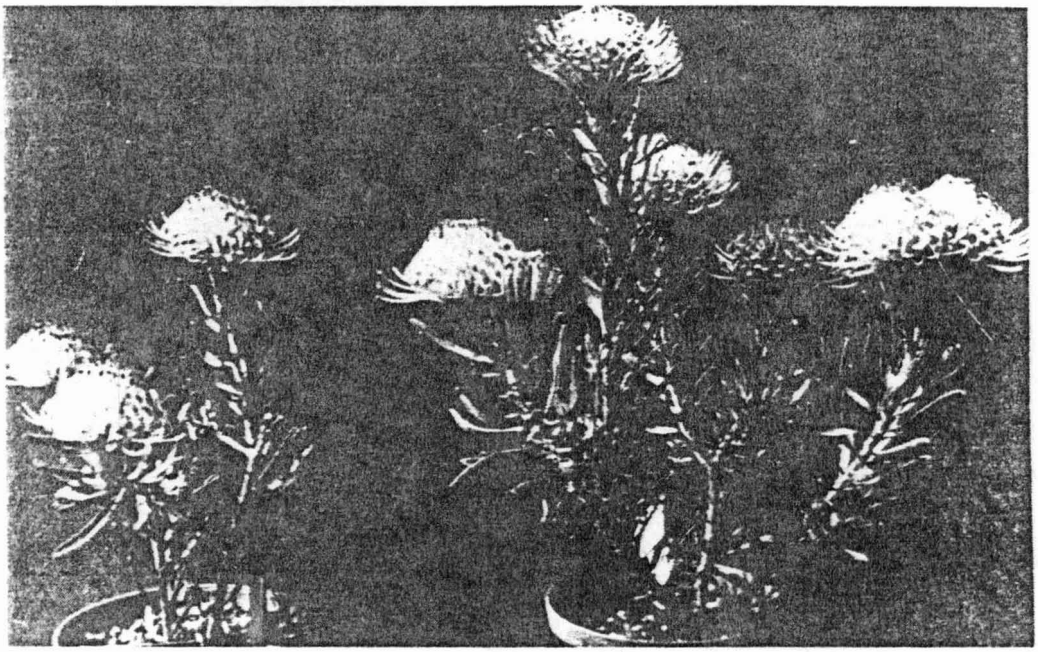

Figure 1. *Leucospermum* "Ballerina' flowering pot plants.

Figure 2. *Leucospermum mundii* flowering pot plants.

on each branch (data not presented). These reach flowering in case of disbudding or abortion of the terminal bud for various reasons. Flowering pot plants of this group can be produced commercially by the use of any of the production systems described.

Group B — Multiple, small flower heads: This group includes several clonal selections of *L. oleifolium* and *L. mundii*, which bear conflorescences (multiple inflorescences, 2 — 4 cm in diameter, grouped together terminally — figure 2). Each conflorescence contains heads which are at several opening stages, and are of different size and color.

1. Branched cuttings, prepared on mother plants of 'Ballerina', 'Tango' and *L. tottum*, rooted very well under all three production systems. The rooting percentages of branched cuttings of *L. oleifolium* and *L. mundii* were significantly lower because the temperature of the rooting media could not be maintained constant during the autumn and winter months. It was therefore difficult to separate the effects on rooting percentage of the physiological status of the cutting (seasonal factor) and of mist bed temperature. Therefore comparisons among species and varieties trialled at different times of the year were unsatisfactory.

2. The use of large branched cuttings, prepared on mother plants, shortens the production time from 17 months to 6 or 8 months (table 2). This provides a considerable saving in growing expenses and produces high-quality flowering pot plants at the time of optimal demand in the European markets (September — April) (Brits *et al.*, 1992).

3. The rooting ability and growth period of *Leucospermum* flowering pot plants differed markedly among clones and among production systems. By using group A *Leucospermum*, it was possible to reduce production time from 17 months (system no. 4) to 6 — 8 months. At the same time, it was possible to obtain high and commercially acceptable percentages of flowering plants, regardless of the production system being used, except in system 3 ('rapid production'), where many of the flower buds dropped during rooting (Brits, 1989b; Brits *et al.*, 1992). Flower bud abortion problems can possibly be overcome by more accurate determination of cutting and harvesting dates, or by harvesting cuttings earlier (system 2), during the late summer, and then realising normal initiation of flowers onto well-rooted cuttings (Brits *et al.*, 1992). Using *Leucospermum* 'Ballerina' it was possible to obtain 100% flowering pot plants within 6 months without any flower bud droppage.

4. Using group B *Leucospermum*, it was possible to obtain a high percentage of flowering pot plants only by the use of the long production method (17 — 19 months — system 4). None of the other production systems tested reduced production time. The low percentage of group B pot plants which flowered in less than 17 months was caused by insufficient thickness of the stems. Usually, branches thinner than 2.5 mm in diameter did not flower (table 2).

5. Efficient growth to produce a well-branched pot plant and a sufficient number of inflorescence buds could not be achieved in 12 months (system 1) from single cuttings (table 2). Even in 'Ballerina', which gave 92% of flowering plants, the average number of buds/inflorescences were unacceptably low. This illustrates the inherent problem of slow growth in the production of woody plants as potted items, in contrast to herbaceous pot plants.

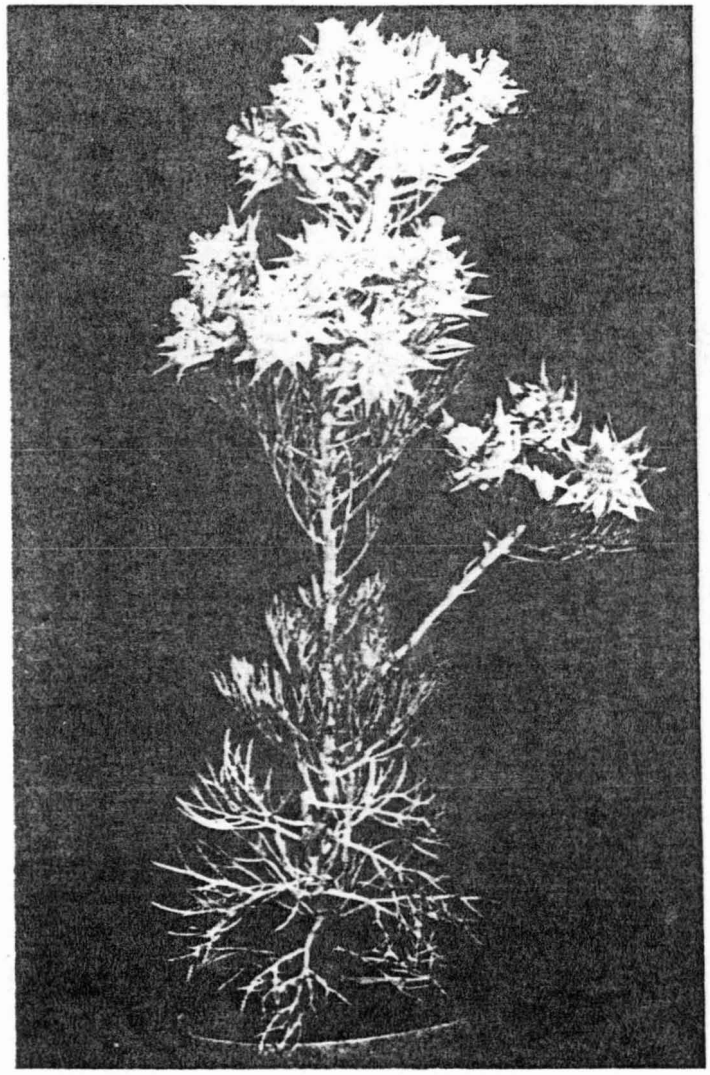

Figure 3. *Serruria* 'Bridesmaid' flowering pot plant.

4.2. *Serruria*

Serruria produces conflorescences of essentially the same type of inflorescences as Group B, except that each inflorescence is borne on a 1 —2 cm peduncle (figure 3). *Serruria* cultivars and selections were tested for flowering as pot plants and the results of these experiments were very statisfactory, indicating the prospect of producing excellent flowering pot plants for export. The low rooting percentage of *Serruria* selections in the winter and spring were probably due to the low temperatures of the rooting medium. A high percentage of flowering plants (75 — 85%) can be obtained within a short growing period (4 — 6 months) by early harvesting of cuttings in the summer (table 4).

Selections were found with a promising shelf life of 30 — 55 days). It was also found that cool storage (at 4 °C and 90% R.H.) in darkness for 4 and 8 days did not lead to damage and quality loss during or following transport (table 5).

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Table 1. Rooting percentage of *Leucospermum* cuttings used for production systems.

Table 1a. Non-induced unbranched hardwood cuttings rooted in spring (system 1 for 10 weeks).

Species/ Hybrid	Code	No of plants			Date stuck	Comments
		Cuttings	Died %	Rooted %		
L. mundi	82 10 04	80	0	84	18/10/90	Temperature at the base of the cuttings was 22 °C
L. oleifolium	85 09 04	74	3	96	18/10/90	
L. oleifolium	85 09 05	45	2	76	18/10/90	
L. tottum	82 10 07	80	0	94	10/10/90	
'Ballerina'	—	301	0.5	86	18/10/90	
'Tango'	—	258	0.4	97	18/10/90	

Table 1b. Non-induced branched, softwood cuttings rooted in summer (system 2 for 5 weeks).

Species/ Hybrid	Code	No of plants			Date stuck	Comments
		Cuttings	Died %	Rooted %		
L. mundi	82 10 04	7	6	66	8/3/91	Temperature at the base of the cuttings was 22 °C
L. oleifolium	85 09 04	25	8	98	8/3/91	
L. oleifolium	85 09 05	100	26	52	8/3/91	
L. tottum	74 11 05	50	18	72	8/3/91	
L. tottum	82 10 07	25	2	60	8/3/91	
'Ballerina'	—	75	12	86	8/3/91	
'Tango'	—	120	2	93	8/3/91	

Table 1c. Induced branched, semi-hardwood cuttings rooted in autumn (system 3 for 12 weeks).

Species/ Hybrid	Code	No of plants			Date stuck	Comments
		Cuttings	Died %	Rooted %		
L. mundi	82 10 04	100	2	6	16/5/91	Uncontrolled temperature at the base of the cuttings was 8 — 18 °C
L. oleifolium	85 09 04	50	58	0	16/5/91	
L. oleifolium	85 09 05	25	84	0	16/5/91	
L. tottum	74 11 05	100	15	74	20/5/91	
L. tottum	82 10 07	100	26	24	20/5/91	
'Ballerina'	—	100	—	75	20/5/91	
'Tango'	—	125	22	50	08/5/91	

Table 2. Growth and flowering of several Leucospermum cultivars as affected by the different production systems.

Code no Cultivar	Production		Date		Average* per plant (cm)			% of flowering	Flowering period
	System no	Time (months)	Rooted	Transplant	Diameter of secondary shoots	Number of inflorescences	Diameter of inflorescences		
'Ballerina'	1	12.0	18/10/90	01/02/91	0.39	1.8	9.4	92	02/10-20/11
	2	7.0	08/03/91	26/04/91	0.34	4.6	9.5	90	05/10-18/11
	3	5.5	20/05/91	20/08/91	0.39	3.5	9.8	100	31/10-10/12
	4	16.5	11/05/90	14/08/90	0.39	3.9	10.1	100	24/09-04/11
'Tango'	1	11.5	18/10/90	01/02/91	0.50	2.0	7.2	66	25/09-23/10
	2	6.5	08/03/91	26/04/91	0.43	2.9	7.0	86	26/09-23/10
	3	5.0	08/05/91	26/09/91	0.42	1.3	9.0	42	10/11-10/12
	4	16.0	11/05/90	14/08/90	0.48	3.4	9.2	100	04/09-18/10
L. totum T82 10 07	1	12.5	19/10/90	04/02/91	0.25	0.2	9.4	10	04/11-29/11
	2	8.0	08/03/91	26/04/91	0.34	3.0	9.5	86	01/11-03/12
	3	5.5	20/05/91	08/10/91	0.28	1.4	9.4	43	25/11-17/12
	4	17.0	06/06/89	14/08/89	0.42	5.5	10.1	100	30/10-04/12
L. mundi T82 10 04	1	11.5	18/10/90	04/02/91	0.29	5.5	2.3	61	01/10-18/10
	2	8.0	31/01/91	18/04/91	0.36	3.5	2.3	43	04/10-25/10
	3	–	16/05/91	Didn't root 29/11/90	–	–	–	–	–
	4	16.0	22/05/90		0.33	12.0	2.6	83	01/10-12/11
L. oleifolium T85 09 04	1	12.0	18/10/90	07/02/91	0.27	0.5	–	9	18/10-21/11
	2	7.5	08/03/91	02/05/91	0.36	0.1	–	10	20/10-26/11
	3	–	16/05/91	Didn't root 14/08/90	–	–	–	–	–
	4	17.0	11/05/90		0.34	8.3	3.8	100	16/10-04/12

*Average of 10 pot plant measurements made on 23/10/91

Table 3. Rooting percentage of *Serruria florida* x *S. rosea* F_1 hybrids (produced from single non-branched cuttings).

System	Hybrid code no/name	Rooting date	Transplant date	Week 10	12	13	20	Comments
				Rooting percentage				
4	'Bridesmaid'	07/2/91	18/4/91	87%	—	95%	—	Inflorescence buds developed in mist bed.
	'Fairy'	07/2/91	18/4/91	67%	—	88%	—	Temperature at bottom heat was 22 °C
2	T89 08 11	11/1/91	10/4/91	—	100%	—		-do-
	T89 08 14	11/1/91	14/4/91	—	88%	—		-do-
	T90 09 12	11/1/91	14/4/91	—	96%	—		-do-
	PLT — 6	11/1/91	14/4/91	—	92%	—		-do-
3	'Bridesmaid'	25/5/91	1/10/91	11%	27%	32%	65 %	Uncontrolled temp. was 8 — 18 °C
	'Fairy'	25/5/91	1/10/91	11%	37%	41%	90 %	-do-
1	'Bridesmaid'	06/9/91	6/12/91		34%	37%		-do-
	'Fairy'	06/9/91	6/12/91		32%	41%		-do-

Table 4. Flowering season and % flowering of *Serruria* hybrid pot plant selections (produced from single non-branched cuttings).

Hybrid code no	Date		Flowering period		% of flowering	Average inflorescences/ plant
	Rooting	Transplant	Dates	Days		
'Bridesmaid'	01/2/91	18/4/91	22/4-08/8	105	85	2.0
'Fairy	07/2/91	18/4/91	17/5-30/8	81	75	3.4
T89 08 11	11/1/91	10/4/91	21/5-20/7	55	80	1.7
T90 09 12	11/1/91	14/4/91	16/6-09/7	24	20	1.2
T89 08 14	11/1/91	14/4/91	01/6-09/7	35	54	2.0
PLT - 6	11/1/91	14/4/91	15/6-04/7	56	70	1.6

Table 5. The influence of dark simulated conditions (temp. 4°C and 90% RH) on flowering and shelf life of *Serruria* hybrid pot plants.

Hybrid code no	Treatment	Shelf		Comments
		Date	No of days	
'Bridesmaid'	Control	18/7-11/9/91	27 – 48	20% of plants sprouting
	4 days	22/7-23/8/91	30	
	8 days	26/7-26/8/91	17 – 30	
'Fairy'	Control	18/7-11/9/91	31 – 55	40% of plants sprouting
	4 days	22/7-11/9/91	52	
	8 days	26/7-11/9/91	38 – 46	

Table 6. The influence of dark simulated conditions (temp. 4°C and 90% RH) on flowering and shelf life of *Leucospermum* pot plants.

Hybrid species code no	Treatment	Shelf life		Comments
		Dates	No of days	
'Ballerina'	Control	07/10-01/11/91	25	
	4 days	11/10-01/11/91	21	
	8 days	15/10-07/11/91	23	
L. oleifolium T85 09 04	Control	01/11-18/11/91	18	Blackening of buds. Discolouration of leaves.
	4 days	04/11-18/11/91	17	
	8 days	05/11-25/11/91	17	
L. mundii T82 10 04	Control	07/10-18/10/91	11	Discolouration of leaves.
	4 days	11/10-18/10/91	7	
	8 days	15/10-25/10/91	10	

SELECTION CRITERIA FOR PROTEA FLOWERING POT PLANTS

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Abstract

Potted flowering proteas are marketable novel products of which the basic production technology has recently been established. The first prototypes have elicited a favourable response from marketing experts. A major objective at this stage is to identify a reasonably broad range of genotypes that will yield good potted items using simple and economic production methods. These methods include both the 8-month "rapid" and conventional 18-month production systems. The objectives of the present study were to select within a range of pot plant candidates those items suitable for shaping shoots on the cutting mother plant using both the above production systems. Candidates were evaluated for field response to:
- chemical branching treatments
- growth retarding treatment with paclobutrazol.
In the nursery phase of production, candidates were further evaluated for:
- ability to root under ambient temperature (no bottom heating) in full sunlight
- disease tolerance.
Generally candidates responded well to growth regulator manipulations in the field. However statistical interaction between genotype and treatment effects was strong, indicating the need to evaluate candidates individually for response to treatment combinations. Although 8-month rapid production was possible with certain selections, other promising material could only be produced successfully over 18 months. The best items were *Leucospermum* 'Ballerina', *L. tottum* (T741206) and *L. mundii* (T821004), all 8-month items; and *L. oleifolium* (T850903) and *Serruria* 'Fairy', 18-month items.

1. Introduction

Traditional cut flowers have lost significant ground to flowering pot plants on the international flower markets over the past 15 years. Presently the production of flowering pot plants is a booming industry to which new products and cultivars are constantly being added. A special potential exists for pot plants of woody species, e.g. azaleas, ericas and proteas, as opposed to herbaceous types, because of their novelty value, hardiness and showiness. Germany currently produces approximately 30 million flowering *Erica* pot plants developed from a single South African species. The size of the European potted market is R2.8 billion per annum while that of the local market is ca. R50 million.

Attractive and unusual flowering pot plant prototypes have been produced from genera of the South African Proteaceae. Although little market research has been done there is a high expectation of the marketability of these products. Protea pot plants can be produced conventionally from single-stem cuttings, over 18 months in the nursery up to market readiness. A "rapid production" method was developed (Ben-Jaacov *et al.*, 1986) consisting of rooting branched cuttings (Brits, 1989). Cuttings harvested during

flower initiation resume flower development after rooting, producing flowering cuttings after 6-8 months. A technique using ethephon spraying has been developed to produce a well-branched *Leucospermum* cutting (primary shoot) on the mother plant in which axillary (secondary) shoots have both a good wide emergence angle of growth and the desired thickness to produce a flower (Brits *et al.*, 1992). An emergence angle greater than can be achieved with manual pinching is aesthetically desireable in *Leucospermum* (Brits *et al.*, 1992) and *Serruria* (unpublished). Paclobutrazol has shown good promise as a chemical shoot growth retardant without unwanted side effects (Ben-Jaacov *et al.*, unpublished). However plants appear to differ in their reaction to treatments.

The rapid production technique requires material specially selected for propensity to flower following rooting (Ackerman and Brits, 1991). A series of protea clones has been identified which yield a high percentage superior flowers when rapidly produced. These include selections of *Leucospermum* with a single large head or inflorescence (*L. cordifolium, L. lineare, L. tottum*); small, multiple heads or conflorescence (*L. mundii, L. oleifolium*); *Leucadendron* (*L. discolor, L. salignum*); and *Serruria* bearing a conflorescence (*S. rosea, S. florida*); and especially interspecies F_1-hybrids of these.

In a recent study good progress was made in economizing the pot plant rapid production technology by 1) the rooting of cuttings using ambient seasonal temperature (without bottom heating); 2) early (mid-summer) rooting of cuttings to utilize the new root system for flower induction (Brits and Ackerman, unpublished). Normal inflorescence development in protea pot plants is strongly dependent on high light levels (Brits *et al.*, 1992) and promising results were found in preliminary tests in which cuttings were rooted in full sun in autumn (Brits and Ackerman, unpublished).

The objectives of the present study were to continue both rapid and conventional 18-month pot plant development by:
1) selection within a range of pot plant candidates for items that respond well to chemical and other methods of shaping shoots on the cutting mother plant;
2) selection for ability to root effectively under ambient summer temperatures.

2. Materials and methods

Ten outstanding pot plant selections were subjected to shoot branching and growth retardation treatments on the land. These treatments showed promise in previous tests.

Treatments: were applied to 3-year old plants and combined in a 2 x 2 factorial lay-out:
1. Present season primary shoots were manually pinched or sprayed with ethephon 960 $mg.l^{-1}$ (full-cover spray) on October 15;
2. Resulting secondary (side-) shoots were sprayed with paclobutrazol 50 $mg.l^{-1}$ on November 30 or left unsprayed.

Replications: All treated shoots were harvested and for each selection 4 treated shoots per treatment were selected randomly from harvested batches.

Responses: the following variables were measured on treated shoots:
1. Number of secondary shoots on a primary shoot
2. Branching angle of secondary shoots relative to primary
3. Length of secondary shoots

4. Diameter of secondary shoots.
Rooting ability without bottom heat in full sun: this was measured separately at 16 weeks for 13 selections and processed with a rooting index percentage method (Brits, 1986). Some selections showed early readiness for harvesting and consequently two rooting dates were used, 1993-02-15 and 1993-03-22. Cuttings were harvested either as branched shoots or unbranched (single) shoots which were planted 3 – 4 per rooting bag.
Disease susceptibility: cuttings were observed for development of diseases.

3. Results and discussion

Selections x treatments interactions were mostly significant (table 1). This is not surprising considering the strong genotypic effect, reflected in the very low probability values for F-values for the effects of selections (table 1). Results are therefore presented for selections individually in the summaries below.

Table 2 presents the effects of branching treatments (manual pinching and ethephon application) on emergence angle and number of secondary shoots per treated primary; and of growth retardant (paclobutrazol) treatments on secondary shoot length and diameter.

Ethephon was effective as a chemical branching agent, confirming previous results (Brits *et al.*, 1992). However ethephon depressed growth in some selections (e.g. *L. oleifolium* T 850904) and these selections should therefore not be treated without additional testing. Ethephon was also effective in increasing the emergence angle of secondary shoots over manual pinching (table 2) as was found for *Leucospermum* 'Ballerina' (Brits *et al.*, 1992). Secondary shoot length was significantly reduced by paclobutrazol in some selections (table 2, e.g. *Serruria* 'Bridesmaid'). Where ethephon caused shoot length reduction the effect was additive to that of paclobutrazol (e.g. *L. muirii* T900601). Number of secondary shoots also increased significantly in some cases (table 2) and therefore reduced shoot length could have resulted from a negative correlation with number. Generally little effect was found on shoot diameter. Rooting percentages under outdoors conditions (without bottom heating) was high for some, but differed greatly between selections (table 2).

A. ROOTED 93-02-15 (table 2)

Leucospermum *conflorescences*

T900601 (*muirii*). This yellow flower selection rooted well and early and reacted excellently to ethephon treatment with respect to length, angle and even increased shoot number. This could be a good yellow flower type if leaf spot disease can be controlled.

T821004 (*mundii*). An early growing selection which was not treated with ethephon, however the plants branched naturally well and with a good angle. Rooting was excellent and rooted plants showed good tolerance to disease. The plant is a relatively slow grower.

T850903 (*oleifolium*). This selection branched well and developed a compact growth, thus eliminating the need for manipulation. Rooting at this early stage led to a high percentage rooting. The flowering response of the 8-month product is not good, therefore this selecton could be suitable as an 18-month pot plant with very attractive

and prolific flowering.

B. ROOTED 93-03-22 (table 2)

Serruria
T840606 (*florida x rosea* 'Fairy'). Secondary shoot length was satisfactorily reduced and angle increased, apparently without growth depressing effects or reduction of shoot diameter. Ethephon + paclobutrazol gave the best results. However rooting response was poor and flowering response is apparently also poor. The cultivar is also susceptible to *Botrytis* infection of young flower buds. Since 'Fairy' was found to perform excellently as pot plant when grown on in the container, it could probably be used as an 18-month product, following standard rooting with bottom heat, to improve its rooting.
T760401 (*florida x rosea* 'Bridesmaid'). This cultivar rooted even poorer than 'Fairy'. Like the latter it is however very attractive and vigorous, responding well to manipulations. It should therefore be tested further as an 18-month pot plant using conventional rooting.

Leucospermum *conflorescences*
T900603 (*oleifolium*). Rooting response of this selection under outdoor conditions was poor.
T850904 (*oleifolium*). Rooting response was not satisfactory. Ethephon treatment appeared to depress growth. The selection may be further compared with other Betty's Bay-type
selections as an 18-month pot plant.

Leucospermum *single inflorescence*
T741206 (*tottum*). Shoot angle increased significantly with ethephon treatment, without side effects. This is the best rooting selection which gave 100% rooting in 6 weeks, repeating the performance of a previous year. Flower development was normal.
T731002 (*cordifolium*). Shoot length of this selection was strongly reduced by both ethephon and paclobutrazol treatment, the two agents acting additively. Rooting was reasonable but flowering response is dubious. Because of its ease of manipulation, its very attractive, tidily arranged small leaves and bright red, small flower heads, this *L. cordifolium* selection could be further tested as an 18-month product.
T771014 (*L. cordifolium x L tottum*). Rooting was too poor to justify further developmental work with this selection.
Totsiens (*L. lineare* hybrid). 'Totsiens' has an interesting flower similar to that of 'Ballerina'. It gave a naturally good shoot angle and reasonable rooting and could be compared further with 'Ballerina'.
T821014 (*L. cordifolium x L tottum*). Rooting was reasonable. This selection could be considered for both rapid production and as an 18-month pot plant.
T821007 (*L. tottum*). With its excellent rooting this "star"-type tottum has good potential as an 8-month pot plant.

4. Conclusions

Growth regulator applications were generally effective over two genera and all inflorescense types tested. Because of strong genotype-treatment interaction the testing of cultivars individually is necessary. Selections responding well to shaping with growth regulators on cutting mother plants are candidates for the 8-month rapid production system. However some of the selections are clearly more suited to an 18-month conventional nursery production schedule. A minority of selections appear to have the characteristics required for rapid 8-month production.

Where selections prove to be suitable only for conventional 18-month production, some degree of manipulation of the cutting harvest whilst still on mother plants may yet be useful. This needs further investigation.

Rooting ability appears to be of primary importance in economic production systems which uses minimal facilities. This emphasises the importance of screening candidates foremostly on the basis of rootability.

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Table 1. Summary of analyses of variance for treatment effects on 10 field-grown progeny test plant selections: branching and growth retardant effects on number of secondary shoots per treated primary shoot, emergence angle of secondaries, secondary shoot length and diameter. Branching treatments were hand pinching and ethephon spraying; growth retardant treatments were paclobutrazol spraying vs. untreated control.

Source of variation	d.f.	Probability of F-value			
		Number	Angle	Length	Diameter
Selections	9	< .001	< .001	< .001	< .001
Branching treatments	1	0.038	< .001	< .001	< .001
Growth treatments	1	0.896	0.831	0.037	0.917
Selections x Branching	9	0.105	< .001	< .001	0.011
Selections x Growth	9	0.239	0.707	0.029	0.028
Branching x Growth	1	0.513	0.655	0.342	0.795
Selections x Branch x Growth	9	0.527	0.280	0.014	0.001
Residual	120				
Total	159				

Table 2. Effects of two branching (manual pinching and ethephon spraying) and growth retardant (paclobutrazol and control) treatments on secondary shoot length and diameter, emergence angle and number of secondary shoots per treated primary, of 10 protea pot plant selections. "Angle" and "Number" values are averages of manual treatments and of ethephon treatments. Three additional selections were partly evaluated. Rooting percentage (16 weeks) of branched shoots are given. Values marked with the same letter within selections (excepting "Rooting") within columns do not differ significantly ($P > 0.05$).

Code	Cv./ Species	Treat-ment	Length (mm)	Diam (mm)	Angle °	Number shoots	Rooting
A. ROOTED 93-02-15							
T900601	*L. muirii*	Man + pac	121b	2.53a	-	-	84.9f
		Manual	163c	2.58a	16.6a	5.12a	
		Eth + pac	74.5a	2.48a			
		ethephon	100ab	2.35a	29.6b	6.37b	
T821004	*L. mundii*	Man + pac	130a	3.45a	-	-	-
		Manual	144a	3.30a	-	-	
T850903	*L. oleif.*	Man + pac	127a	2.55a	-	-	80.8f
		Manual	124a	2.58a	-	-	
B. ROOTED 93-03-22							
T840606	'Fairy'	Man + pac	168a	2.65a			32.8b
	S. florida	Manual	153ab	2.65a	17.4a	3.13a	
	x S rosea	Eth + pac	124c	2.63a			
	ethephon		128bc	2.60a	26.1b	3.76a	
T760401	'Bridesmaid'	Man + pac	154ab	2.50a			-
	S. florida	Manual	172a	2.50a	15.9a	2.75a	
	x S rosea	Eth + pac	128b	2.13a			
		ethephon	142b	2.30a	25.4b	3.38a	
T900603	*L. oleif.*	Man + pac	135a	2.90a			11.4b
		Manual	102b	2.65a	21.4a	3.38a	
		Eth + pac	104b	2.75a			
		ethephon	150a	2.78a	32.1b	4.88b	
T850904	*L. oleif.*	Man + pac	91.7a	2.98a			40.5cd
		Manual	119b	3.18a	26.8a	4.50a	
		Eth + pac	91.2a	2.93a			
		ethephon	87.7a	2.83a	28.9a	3.63a	
T741206	*L. tottum*	Man + pac	127a	2.13a			100.0
		Manual	123a	2.38a	31.3a	6.63a	
		Eth + pac	103a	2.05a			
		ethephon	108a	2.05a	37.9b	6.00a	

Code	Cv./ Species	Treat-ment	Length (mm)	Diam (mm)	Angle °	Number shoots	Rooting
T731002	*L. cordif.*	Man + pac	158a	4.43a			67.7e
		Manual	161a	5.25b	25.8a	3.63a	
		Eth + pac	90.7b	4.23a			
		ethephon	107b	4.10a	37.4b	3.38a	
T771014	*L. cord.*	Man + pac	133ab	3.58b			34.5bc
	x L tott.	Manual	158a	3.80b	19.8a	3.13a	
		Eth + pac	116b	3.10a			
		ethephon	113b	3.13a	32.3b	3.75a	
T741102	'Totsiens'	Man + pac	84.3ab	3.30b			61.3ce
	L. lineare	Manual	62.2b	2.85a	34.9a	3.00a	
	hybrid	Eth + pac	103a	2.78a			
		ethephon	77.3b	3.00ab	29.3a	3.88a	
T821014	*L. cord.*	Man + pac	148a	4.88b			67.3def
		Manual	144a	3.85a	29.1a	3.50a	
		Eth + pac	126a	3.75a			
		ethephon	147a	3.75a	28.4a	3.75a	
T821007	*L. tottam*	-	-	-	-	-	95.7f

CROP SCIENCE OF PROTEACEAE IN SOUTHERN AFRICA: PROGRESS AND CHALLENGES

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Abstract

Cultivation of proteas as agricultural products is fast approaching maturity with production of cultivars becoming a reality for many of the more than 30 products. Although standard crop science practices can to some extent be used with success within product ranges, Proteaceae is quite successfully living up to its name, with uncooperative selections appearing in almost every sphere of production and export. Worldwide Protea growing experiences increasing problems with the horticultural requirements not only of types or species, but of every individual cultivar or selection. The extent to which our present crop science knowledge satisfies their needs and the areas requiring research are investigated. New trends in protea growing technology are highlighted.

1. Propagation

Current propagation techniques for Proteaceae and research priorities were recently reviewed (Malan, 1992). This report will therefore only highlight subsequent development.

Seed: Seed is still widely used for the establishment of varieties not represented by cultivars and increasingly for the establishment of foliage plantations. The decline in research reports on seed germination techniques for commercial Proteaceae varieties probably reflect the increased availability of cultivar material and/or that agriculturally satisfactory seed dormancy breaking techniques had been established for *Protea*. Seeds are incubated at 5 — 12 °C for 20 to 40 d (pretreatment in water at 50 °C for 30 min, dried, dusted with Dithane and germinated in sand or between wet bags) (Meynhardt, 1976; Brown and Van Staden, 1973). Dormancy can also be broken in *Leucospermum* (8 min concentrated H_2SO_4, 24 h soak H_2O_2 plus promalin, germination in sand) (Brits, 1986a) and *Leucadendron* (dependent on seed type either as in *Protea* or *Leucospermum*) (Brits, 1986b). The research on harvesting stage, seed disinfection, viability testing for commercial use and dormancy breaking techniques for other currently non-commercial genera (*Mimetes, Orothamnus*) are still important areas for future research.

Cuttings: Rooting of cuttings of most commercial selections and hybrids is (at Elsenburg) no longer considered difficult when using a standard technique i.e., 20 cm semi-hard wood cuttings treated with 4 000 mg/dm^{-3} IBA in a sand: peat(organic): polystyrene medium with 23°C bottom heat and receiving hourly mist for 1 to 5 min. during daylight hours. This is mostly due to more precise harvesting of cuttings according to physiological developmental stage, better sanitation practices and better general management of the nursery. Cuttings of a few species e.g., *P. longifolia, P. holosericea, P. magnifica* are however still very slow to root. Unless research indicates it to be the result of inherent rooting characteristics, it may necessitate the

indicates it to be the result of inherent rooting characteristics, it may necessitate the development of at least one more propagation system. The situation requires further attention. The current system which ensures a high success rate is also rather expensive. The development of a cheaper system e.g., without bottom heat for easier-to-root cuttings, cheaper rooting material and more effective use of space are areas which require attention. Manipulation of cutting mother plants, an essential to reduce mother plant plantation size has not been well researched or documented and require more attention.

Grafting: VOPI released its first *Phytophthora cinnamomi* tolerant *Leucospermum* rootstock cultivar during 1992 and grafted plants can now be ordered. Grafting is often reported to be easy, and that it can be readily applied even between some genera. The commercial use of the available techniques have been found to be either insufficiently successful or prohibitively expensive. Commercially acceptable techniques need to be developed. Rootstocks with problem related characteristics (lime tolerance, fast rooting when needed for cutting grafting, low phenol content) need to be developed for the other genera. The area-specificity of many proteas is evidently largely related to soil adaptability and rootstocks with better allround adaptability to different soils also need to be developed for all genera.

Tissue culture: The potential of tissue culture to rapidly multiply new Proteaceae selections/hybrids has in the past probably been over estimated. The main reasons are: 1. the high cost and risk of success involved in technique development; 2. the availability of synthetic axillary bud sprouting agents (benzyladenine, ethephon) to increase cutting production on cutting mother plants; 3. a longer pre-production period of *in vitro* produced plants; and 4. a relatively small (1 000 to 20 000) annual requirement for Proteaceae plants per selection/hybrid. This does not however nullify the quarantine acceptance of TC plants, its role in rejuvenation of physiologically aged material, its use in physiological studies, etc.

2. Establishment

The decision on establishment technique/system is financial, resulting in products with different commercial value being established using different material and techniques. Success rates in the field establishment of proteas vary enormously and probably present as many unanswered questions as there are techniques/systems of establishment.

Seed: The establishment of lower income dried flower, foliage products and cut flower products exhibiting very little natural variation are done by sowing of large amounts of seed directly into prepared field rows or lanes. Also the establishment of non-utilized species for evaluation as new products for uncertain markets and value may not warrant higher input cost. Seed/soil disinfection treatments, pre-sowing viability testing to determine sowing density, pre-sowing germination treatments, season/timing of establishment, sowing techniques and post germination seedling wilt disease control are just some of the new fields of study which require attention.

Seedlings: A number of higher income products are not yet represented by cultivars/selections and are still commercially grown from seed. The seedlings may be transplanted directly from germination beds to the field at the 2 to 6 leaf stage, but are often transplanted earlier to nursery bags and grown on for 6 to 9 months under

nursery conditions. These are traditional propagation techniques and there does not seem to be any problem with its application. Such plants are subsequently established in the same way as cuttings, discussed below.

Cuttings: In South Africa, cuttings are generally transplanted to the soil within 4 to 8 months from setting, dependent on the seasons of propagation and rainfall. Soil preparation is related to type of soil, but soils are generally ploughed to a depth of at least 60 cm. The rooting bag is removed with as little root disturbance as possible and the rooting medium buried in the ground. A 50 micron polyethylene or an organic mulch is sometimes used to prevent burning of cutting stems by soil surface, control weeds or conserve water under dryland conditions. Success rates of 0 to 100 percent is attained. The causes of loss can often be traced to factors such as drought, collar rot, heat waves, plant suppression by weeds, incompatibility of rooting/potting medium and field soils etc. Death of a large percentage of plants is mostly attributed to inadaptability of a variety to soil or environmental conditions, but even then it often happens that some of the survivors will perform very well. With seedlings this is more easily explained, but when a clonal orchard is established soil/environment can no longer be blamed. This is an area of crop science which require much closer attention to increase success rates and ensure continued encouragement for prospective growers.

3. Training and Pruning

The traditional belief is that proteas should not be pruned during their pre-production years to allow the plant time to establish itself. This may very well be true when plants are grown in very dry, poor, deep sandy soils where it may take up to two years for plants to start giving significant above-ground growth. The period to first production and the quality of these flowers, but mostly plant architecture and the length of the productive life, can be greatly improved by training and pruning.

Pruning is a growth manipulation, controlling plant architecture and balancing the quality and quantity of saleable flowers produced per plant (Brits *et al.*, 1986). In proteas, quantity is controlled during annual harvesting through planning the number of subsequent shoot production sites available per plant. Two pruning cuts are used, i.e. thinning (decreasing plant complexity - less but longer shoots) and cutting back (increasing plant complexity - more but shorter shoots). Three types of axillary shoots are formed in reaction to cutting back: 1. flowering shoots, 2. vigorous non-flowering shoots (mostly in *Protea*) and 3. short thin non-flowering shoots. Proleptic shoots are formed distally on the flowering stem in many selections/types/species. *Leucadendron* foliage plants also produce thin sylleptic shoots along most of the length of the flowering shoot. The quantity and quality of shoots produced on every bearer is dependent on inherent plant characteristics, plant vigour, time of pruning, position of the cut within the flush and the vigour of the bearer.

Leucospermum: Although seedling plantations are now rather the exception than rule they are still established and should be treated correctly. Seedlings tend towards complexity and failure to reduce complexity following the first growing season will result in most varieties taking a year longer to start producing saleable stems. First year growth generally consist of up to 10 shoots. Training consists of the removal of the weaker shoots and cutting back of the remaining 3 to 5 shoots, to leave 10 to

15 cm stumps (bearers), at the end of the first dormant season. Situation and variety specific guidelines must however still be established.

Leucospermum plants grown from cuttings tend to produce less, but stronger shoots than seedlings, which generally exhibit a sprawling growth habit. If the plants are not trained prior to the second growing season the crawling habit will persist and shoot length will decrease. Training consists of shoots being thinned at the end of the first dormant season to leave 2 to 5 shoots dependant on plant vigour and variety. The remaining shoots are cut back to leave 10 to 15 cm bearers. In both instances if the shoots remaining on the plants are shorter than 10 cm only their tips are removed.

Leucospermum bearing stumps produce 1 to 5 shoots. The flowers on the longest, thickest shoots reach anthesis first. These are also the best shoots to act as bearers the following season. The first flowers are therefore picked with cutting back cuts i.e. 10 - 15 cm of the shoot base is left on the plant until sufficient bearers (estimated number of flower bearing shoots per plant divided by the number of shoots per bearer) are available. All subsequent flowers are picked using thinning cuts. Short, thin non-flowering shoots are removed with thinning cuts prior to or immediately following harvest.

Protea: Actively growing shoots exhibit strong apical dominance. The differences in growth habit between types determining pruning severity are the length of individual flushes and the ability of its apical meristems to retain apical dominance and control. In some *Protea* varieties apical control is lost by just about every flush (*P. repens, P. burchelli*) seemingly dependent on the time of the year. In these plants complexity need to be limited through training. In others (*P. compacta, P. cynaroides*) dominance is retained even to the extent that only one shoot will develop. In these plants complexity need to be increased by pinching the tip of the shoot.

The time when the flowers of the first crop will be initiated (which is not necessarily the first period of flower initiation) will also play a very important role in determining the timing and number of training treatments. Very limited information is, however, available on flower initiation in *Protea*, a subject requiring urgent attention. Guidelines for the severity, timing and number of training treatments need to be established for every variety.

Once training has been successfully done, *Protea* harvesting and pruning are essentially the same process, with flowers with sufficiently long stems being harvested by cutting back and those with short stems with thinning cuts. Non-flowering weak shoots are retained. Care must be taken not to leave any unharvested flowers or their stems or very vigourous non-flowering shoots on the plant, as this leads to poor plant architecture.

Leucadendron: The commercial types can be divided into multi- branched foliage and single stem cut flower products but most growth habits in between are also represented. Seedlings and even mature foliage products exhibit weak apical dominance but mostly strong apical control causing them to tend toward complexity. Training is essential to shorten the pre-production period and to control the quality vs. quantity of production. The very complex *Leucadendron* plants (*L. floridum, L. conicum, L. coniferum*) do not respond well to extreme pruning practices controlling their architecture. This may be due to their axillary sites being spent as a result of poor apical dominance and weak sylleptic shoots present on retained bearers taking more than 1 season to assume dominance. Improved pruning practices need to

be established for plants with such growth habits.

Single stem products exhibit a simplistic growth habit with strong apical dominance and mostly also strong apical control, with training only designed to prepare an acceptable production structure for the plant. During harvesting, sufficiently long flowering shoots are all cut back leaving a 10 - 15 cm stump with shorter flowering shoots harvested with thinning cuts. Later in the plants productive life, thinning cuts may also be introduced to longer shoots to increase average shoot length per plant. Weak non-flowering shoots are retained as they will produce the subsequent seasons early crop.

Seasonal crop prediction models accommodating plant type, age and vigour need to be developed for all genera and types to determine the annual ratio between cutting back and thinning cuts.

4. Irrigation

Systems - The irrigation system utilized is related to crop potential, type of production system, the availability of water, the extent of weed infestation and type of soil. It is generally believed that overhead irrigation is not suitable for bearded proteas, but very successful orchards exist which are irrigated in this way. The widely differing crops, environments, soil types and situations probably make research on irrigation systems economically unrealistic.

Requirements - The large variety of plant and soil types and environmental conditions utilized in the cultivation of proteas and the enormous natural inherent variation within and between types makes any kind of general statement almost impossible. It also makes an approach to irrigation requirement research extremely difficult and may cause the results of such work to be of limited value. All this may be true purely because no research has been done and even current guidelines are drawn from information obtained from other crops. It has been established through commercial use that irrigation of proteas will, under certain conditions, significantly improve establishment percentage and increase the quality and quantity of production of many varieties. In some varieties (*P. repens, P. salignum*) the increased growth is often axillary shoots growing past the flower making flowers much more expensive to prepare for export or even totally unmarketable. Proteas' main root growth period is in winter and the inability of winter rainfall variants to adapt to summer rainfall cultivation may be a lack of winter irrigation. I believe irrigation research, if attempted, should be cultivar specific and its main aim should be to determine seasonal water consumption (crop factor) for such cultivars.

5. Soil requirements and fertilization

Although many proteas occur naturally on poor, sandy soils many will grow very successfully on soils containing a clay/loam fraction from 30 to 50 % provided the soil is well drained. Some proteas e.g., *P. mundii, P. lepidocarpodendron, P. cynaroides, Lsp. formosum, Lsp. glabrum, Lcd. salicifolium, B. ericifolia* are adapted to, or may even prefer slightly wetter/shallower soils. The adaptability of such varieties to heavier and poorly drained soils should be quantified to allow for better farm planning. Proteas, like any other plant do need nutrition as is indicated by the fact that they

perform very poorly or die if none is supplied. Excessive availability of nutrients will however also lead to either luxurious production of vegetative growth with no flower production or in severe cases death.

Research on protea nutrient requirements have been primarily focused on the inability of proteas to tolerate normal agriculturally acceptable phosphate and potassium concentrations (15 publications). The current conclusion is that some proteas will actually perform very well if these elements are supplied, but it depends on these elements being accessible only in reasonable quantities. This accessibility is however related to soil physical characteristics such as the adsorption/absorption ability of soil particles, the quantity of the absorbing particles, the organic material content of the soil and the inherent status of these nutrients in a soil. The many extraction techniques available to determine available levels, and types of fertilizers to supplement it, cause the subject to become extremely involved. In the process you get lost and never get to protea fertilization.

For agricultural purposes it needs to be determined at which nutritional level the plant will give optimum productive growth and standards need be established to monitor these levels through leaf samples. Once the optimum leaf nutritional levels (physiological age, shoot position related) have been established fertilization requirements related to soil and fertilizer types can be investigated. In perennial crops annual leaf and soil sampling are subsequently used together to maintain optimum nutrient availability.

A lack of knowledge on standards for leaf analysis for proteas is probably related to analysis of protea material not always reflecting deficiencies/toxicities (Parvin, 1986) even when symptoms are evident on the leaves. It may also be because between type/variant/seedling variation is often higher than variation leading to nutritional abnormalities within types making optimum level determination extremely difficult when working with non-clonal material.

6. Harvesting

Leucospermum: Flowers can be harvested at 33 - 50% styles reflexed resulting in effective style opening and optimum travel ability and shelf life. The market is, however, increasingly demanding a flower with 100% styles reflexed, resulting in a considerably poorer travel ability and shorter shelf life. The reason for this change in consumer demand must be investigated and the problem addressed to prevent a related drop in demand.

Protea: Soft-tip stage, except for *P. mundii, P. lacticolor ,P. aurea* which are harvested at the emergence of the styles.

Leucadendron single stem: Only during the period that it satisfies its role. Poorly coloured Safari Sunset, for instance, will harm the product's market position.

Leucadendron foliage: Any time subsequent to leaf maturity.

Serruria: Second inflorescence anthesis, the terminal inflorescence is removed in the packshed.

7. Post-harvest handling

With leaf blackening being the major problem in post harvest handling of protea

flowers, many of the associated processes and methods of control have been investigated resulting in approximately 50 scientific publications. Current belief is that carbohydrate depletion of leaves through respiration/translocation is the main cause of leaf blackening (Bieleski *et al.*, 1992), and that increasing initial carbohydrate supply through high light intensity treatment are likely to alleviate the problem. Many practical aspects which may influence leaf blackening e.g. time of harvesting, time from harvesting to packshed, the presence of free water on flower and leaves at the time of harvesting, physiological stress levels (irrigation and water stress) prior to and at the time of harvesting, the amount of water the flower must absorb in the packshed or not, to be cooled faster, water quality in the packhouse, cooling temperatures, etc., have been indicated by producers to influence post harvest life, are rather poorly documented.

8. Flowering

Flower initiation in *Leucospermum* (Malan and Jacobs, 1990), *Leucadendron* (Ben-Jaacov and Kadman-Zahavi, 1986) and *Serruria* (Malan and Brits, 1990) have been indicated to be controlled by daylength and can be manipulated through daylength control. Although this may have far reaching implications in Proteaceae pot plant production and perhaps even in protected cultivation, its commercial use in field plantations is, however, still prohibitively expensive. This is largely due to the high light intensity (20 - 30 $\mu E.m^{-2}.s^{-1}$) required to interrupt the natural dark period to elicite a response. The mechanism involved in *Protea* flower initiation is not yet understood. Although clonal commercial orchards has indicated that flowering time can be manipulated through pruning, exact techniques to favourably manipulate flowering time still need to be developed. The flowering time of *Leucospermum* can be extended by disbudding (Brits, 1986c), the extent being dependent on variety.

9. Pot plants

Although the development of 14 to 20 month old Proteaceae flowering pot plants (multi-branched cuttings, pruned and transplanted to 12 cm pots following rooting, grown on for a single growth cycle and then naturally or artificially induced to flower) can be readily produced (Brits *et al.*, 1992), leaf drop on 12 to 14 month old wood results in unsightly plant bases. The long production period would probably also make the plants too expensive. An alternative system e.g. rapid (8 - 12 months) production system, where plant vegetative structure development is completed on the mother plant, and only reproductive development occurs following rooting, seems feasible, but problems are still being experienced with structure development on the mother plant as well as fertilization, irrigation and disease control in the rooting and flower development stage.

Although most proteas can be grown and commercially utilized with relative ease without consideration for general crop science aspects, the resultant product is generally of poor quality and orchard life limited. From the above it is clear that, to intensively cultivate proteas and obtain optimum yield and product quality and orchard life, many aspects of crop science still present daunting challenges.

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ARCHITECTURAL STRUCTURE OF TWO SPECIES OF *PROTEA* GROWN IN SOILLESS CULTIVATION

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Abstract

Experience with soilless farming, with numerous vegetable, arboraceous or floral species, allows us to state that this technique does not modify in any way, the proper architectural features of the species. However, it can cause considerable changes in quantitative aspects of their development, if the nutrition-substrate system is well adapted.

In order to develop proper techniques for cut flower production, we studied the ramification (branching) and flowering processes of seedling populations of *P. eximia* and *P. neriifolia*.

The results of these studies enable us to determine :
- the pruning and harvesting methods that are most adapted to architectural features of the species (for instance, acrotony);
- with some degree of precision, the level of physiological maturity for flowering.

1. Introduction

The production of Proteaceae can be part of horticultural diversification in the south of France, since climatic analogies exist between this region and the native areas of this family. Unfortunately, Proteaceae members used in floriculture (indigenous to Southern Africa) are not sufficiently frost-resistant to tolerate the winter climatic conditions of southern France. The acclimatization trials, as realized in botanical gardens, confirm this. Moreover, Proteaceae are not adapted to limestone, and most soils in the south of France are calcareous clay soils.

Thus, if we want to grow Proteaceae in this region, we must grow them under soilless greenhouse conditions. This is an expensive way of cultivation, but the higher costs incurred by soilless greenhouse cultivation must be ofset by an increase in production and quality, as it is generally observed for other plants.

To increase production requires a good knowledge of the growth and development of these plants. They are not modified by the soilless cultivation in principle but only in their potential expression. Thus, pruning techniques should be modified compared to those plants grown in the open field.

The objective of the experiments was to determine, by studying the architectural structure of two species of seed originated plants and grown under soilless greenhouse conditions, the development and flowering characteristics resulting from this type of culture and also from the different pinching/pruning treatments.

2. Materials and methods

2.1. Materials

Two *Protea* spp. were chosen, because of their level of complexity observed in preliminary trials. They are, from the simplest to the most complex: *Protea eximia* and

P. neriifolia. Seeds of *P. eximia* were sown in a temperate climate glass-house (15 °C) in January 1989 and seeds of *P. neriifolia* in January 1990. The seedlings were transplanted one month later in pots (8 cm across) and put in place at the beginning of June.

2.2. Methods

Plants of *P. eximia* were arranged into 12 blocks, according to Fisher, with 6 plants per plot (density: 2.2 plants.m^{-2}). There were 3 treatments: T1 = the plant has only one axis (order 1) until the first flowering; T2 = the plant is pinched on the axis of the 1st order after the 3rd growth unit (GU); T3 = the plant is pinched on the axis of the 1st order after the 5th GU. For T2 and T3, the axis of 2nd order obtained by pinching the 1st order were deshooted when necessary.

Plants of *P. neriifolia* were arranged into 8 blocks, 1 plant per plot and 3 treatments: T1 = free form; T2 and T3 were the same as in *P. eximia* trial. Two fertigation frequencies were applied for *P. neriifolia*: F2 = 2 x F1.

The term of growth unit employed here is similar to "flush" but indicates more exactly the part of stem included between 2 ranges of scales.

The substrate used was porphyry from the Esterel Mountains (Rhyolitic stone) with a granule size varying between 5 and 8 mm. Night time temperature was set at a minimum of +5 °C during the winter and the day ventilation started when temperature rose above +20 °C. The composition of the nutrient solution is indicated in table 1. Calcium and magnesium traces were present in the tap water.

Table 1. Composition of the nutrient solution.

	N	$PO_4H_2^-$	K	Ca	Mg
Concentration (mM)	0.6	0.1	0.4	2.9	1.9

Micronutrients came from a commercial solution and iron from chelating agents. The amount supplied was 0.5 litre/plant/watering. The frequency (F1) was 4 waterings a day in winter and 8 in summer.

The measurements were made monthly and involved:

- the number of growth units (GU)/plant or/axis,
- the number of ramifications,
- the number of flowers/plant/month,
- the number of GU necessary/axis to have a flower.

3. Results

Before giving our results we should mention how these two species grow naturally (Muller, 1989; Salaün, 1990). Growth is rhythmic and plants are composed of repeated growth units (Hallé, 1979). These growth units, formed inside a bud during the meristem cycle, are produced during an elongation cycle which occurs between two periods of rest. The ramification (branching pattern) is subapical, with orthotrope

direction and occurs when the terminal flowering bud is initiated. The development is said to be acrotonic. This type of architecture is close to LEEUWENBERG's model (figure 1).

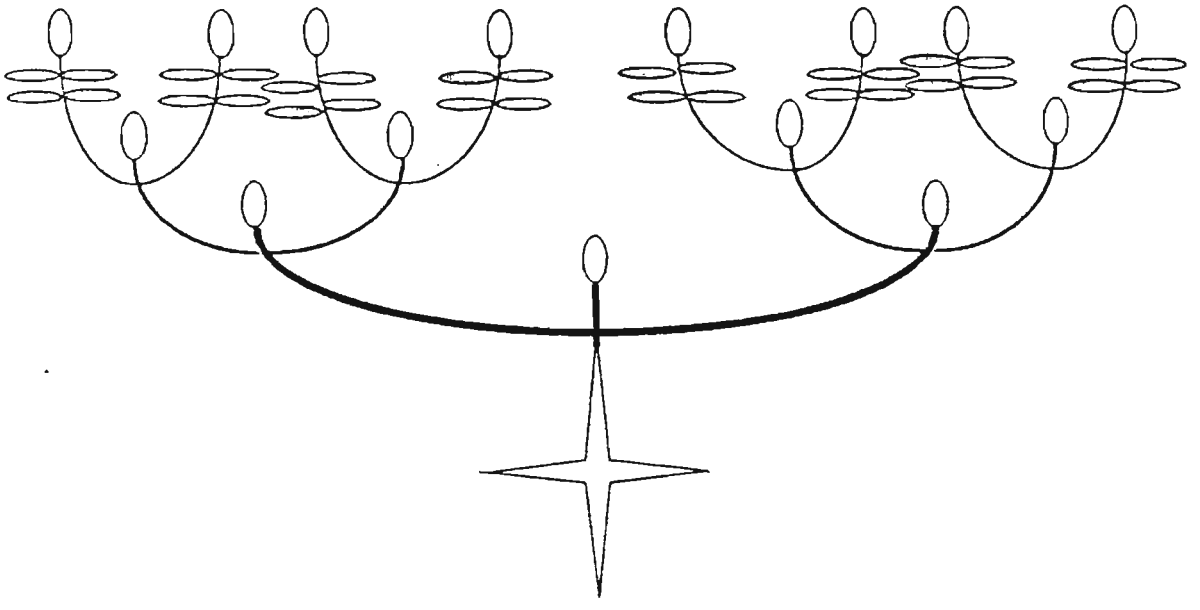

Figure 1. The LEEUWENBERG's model.

3.1. *P. eximia*

The results obtained concern :

- the number of axes produced for a given order according to the level of pinching,
- the number of GU necessary to obtain a flower on a given axis,
- the number of flowering axes obtained for a given axis according to the treatments.

The comparisons have been made to characterize the different potential of expressions depending on the pinching/pruning treatments during a given time of cultivation. The study lasted 36 months, from explanting in June 1989 to July 1992.

An analysis of variance was made to determine the effects of the treatments on the number of axes of the 2nd and the 3rd order (table 2).

Table 2. Comparison of average numbers of ramifications according to treatments.

Order of axis	T1	T2	T3
2	7.65 a	2.99 c	5.47 b
3	3.91 d	9.55 e	9.69 e

For each order and treatment, the values followed by the same letter are not significantly different.

The plants of T2 were pinched in October 1989, 5 months after explanting, whereas the plants of T3 were pinched in March 1990, 10 months after explanting. The plants of T1 were pruned after flowering from November 1990 to March 1991, that is 18 to 22 months after explanting. According to these data, it appears that the potential of expression of ramification of the 2nd order are higher if the plant is older. On the other hand, for the ramifications of 3rd order, the values obtained from T2 and T3 became similar and the values obtained from T3 became higher than those obtained from T1. These results should be carefully considered as the periods of study were shorter for T1 than for the branches of 3rd order.

The level of flowering, estimated at flowering maturity on the different orders of axes in each treatment, gives an average number of GU (table 3).

Table 3. Average number of growth units on flowering axes.

	T1		T2		T3	
Order	Mean	Stand. dev.	Mean	Stand. dev.	Mean	Stand. dev.
1	9.9	1.00	—	—	—	—
2	6.9	1.22	7.3	0.65	5.2	0.53
3	5.3	0.82	6.6	1.03	3.8	0.48
4	—	—	4.6	0.57	—	—

There is no mean value for the 4th order of T1 and T3. During the summer of 1992, the plants of T3 were used in trials involving partial destruction of the branches. After flowering, the axes of the 1st order of plants of T1 were pruned to the 5th GU, whereas the axes of 3rd order of plants of T1, T2 and T3 were pruned to the 1st GU. The analysis of variance and Sheffe's test (table 3) show that the obtained means are significantly different (5% level). Therefore the treatments are significantly different for a given order and among the orders for a given treatment, except for the 2nd and 3rd order of treatment 2.

From this table, it appears that the number of GU necessary for flowering, decreases with an increase in the order of ramification whatever the treatment may be. Furthermore, if we add up the numbers of GU on the different axes leading to bud initiation (taking into account the pruning of the flowers), we obtain a practically constant value of 11 ± 1 GU. These results will allow us to define methods of production.

Finally, regarding the number of flowering axes for a given order, we took two different periods of time into account because of the gaps resulting from the destructive treatments of the summer of 1992. Therefore we compared :

1) the axes of the 2nd order of
- T1, T2 and T3 from explanting to June 1992,
- T1 and T2 from the explanting to April 1993,

*2) the axes of the 3rd order of
- T1 and T2 from explanting to April 1993.

The results are given in table 4.

Table 4. Number of produced flowers/plant according to the orders of the axes and the treatments.

Period	Order	Number		
		T1	T2	T3
06/89 — 06/92	2	2.62 a	2.68 a	3.77 b
06/89 — 04/93	2	6.25 b	2.78 a	—
	3	2.99 a	7.07 b	—

For each period and order, the values followed by the same letter are not significantly different.

Among the different treatments, we observed that for the 2nd order and the 1st period, only T3 is more floriferous whereas T1 had a total number of branches much higher than the two others (cf. table 2). This can be explained by the fact that T1 had a shorter period of time to show its potential of flowering. If we consider the same level of order of axis and the second period, which finishes one year later, the number of flowers produced by plants of T1 is twice higher T2. The contrary was observed from the 3rd order, probably due to the same causes than that of the first period. It is necessary to wait for the production of 1993 — 1994 to estimate floriferousness of T1 on the stems of the 3rd order.

On the other hand, if we consider the number of flowering stems relative to the total number of stems, we obtain, considering the axes of the 2nd order, 82% for T1, 93% for T2 and 70% for T3 whereas, when we consider the axes of the 3rd order, we obtain 76.5% for T1 and 74% for T2.

These first results suggest that :

1) The potentialities of expression of ramification are on two levels which can act in opposition:

- on the first order axes, the more developed the plant is at the time of pinching or pruning, the more branches it produces.
- beyond the 2nd order, the higher the order of ramification, the smaller the number of branches per axis becomes.

2) Similarly, the higher the order of ramification, the smaller the number of growth units needed for flowering becomes. Without confirming the effect of the addition of the number of GU on the axes of successive orders (though we have a relatively constant value of 11 GU), it is possible to state that plants will only flower if the total number of GU is included within a more or less limited interval. We can consider that, from an order n, (not reached in this trial) only one GU on the ramification will become necessary for flowering. It would be advisable to prune back to a lower level when the stem length of flowers starts to decrease. Another possibility would be that a reiteration brings back juvenility to the ramification process (Edelin, 1986).

* From the third axis, the floriferousness seems to be only partly influenced by the cropping system, meaning that a shoot has a stable potential to flower. Therefore, the floriferousness of a plant is a function of the number of branches produced since the ratio of flowering branches/vegetative branches is relatively constant.

3.2. *P. neriifolia*

The results concerning *P. neriifolia* involve a comparison between 3 types of cropping systems. In this study, all the plants have not yet reached a similar

physiological age during the experimental period (June 1990 - December 1992). Therefore, it is only possible to suggest trends, especially since the variability and the limited number of plants lead to results that are, most of the time, not significant.

Figure 2 shows the potential of producing branches as a function of the orders and the treatments.

It shows that the number of branch production by a given axis decreases according to its order. In other words, the higher the order of branches is, the lower the degree of branching is. However, during the considered period, the branches of the 3rd and the 4th order had not reached their full potential of expression. The frequencies of fertigation did not affect the growth of the plants.

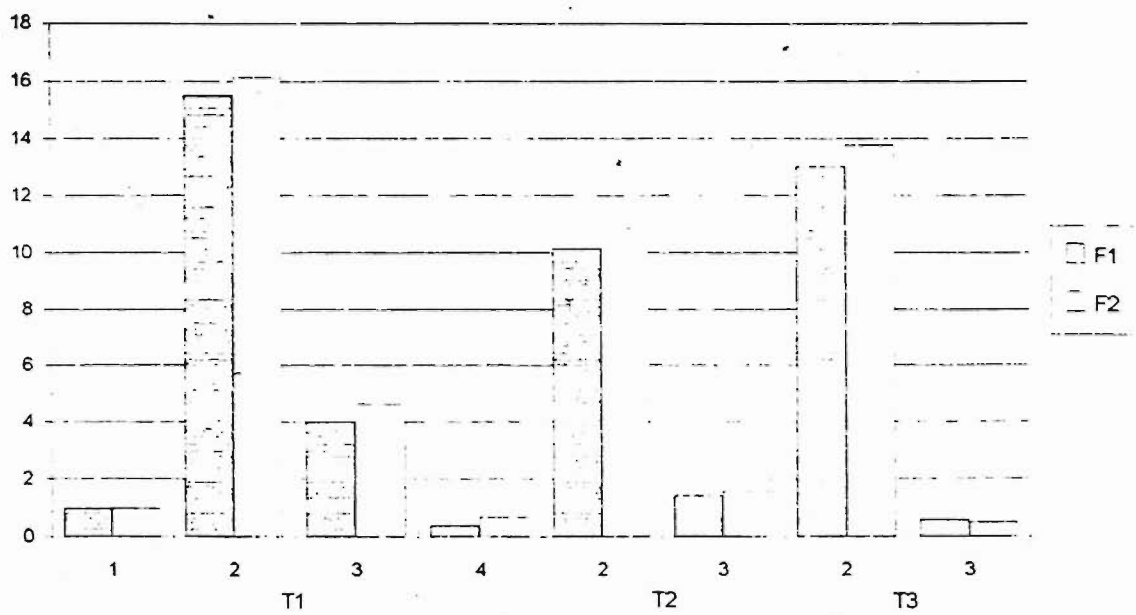

Figure 2. Number of ramifications according to the order and the treatment.

Considering the number of GU necessary to flowering, it appears that in plants of T1, as the order of ramification becomes higher, smaller numbers of GU are needed for bud initiation (figure 3). A comparison between the axes of order 2 of T2 and T3 shows that, in order to flower, plants of T3 require less GU than those of T2. This can be explained by the addition of GU (T2 = 3+6; T3 = 5+4). Here again, the frequencies of watering had no significant effect.

Finally, there is no significant difference in the number of flowers among identical orders of different treatments. Here again, the results should be carefully considered since, during the time of study, all the branches had not reached their full potential of expression. Here, the fertigation frequency has an important effect. If we add the number of flowers produced (figure 4), we obtain a number for the highest frequency that is almost twice that of the lowest one.

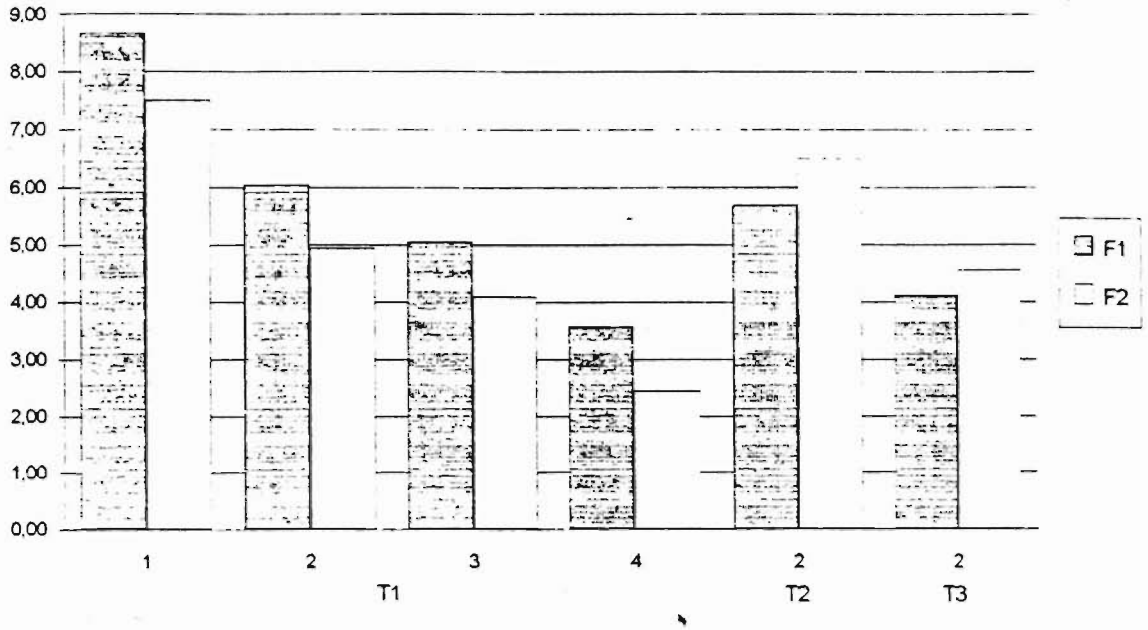

Figure 3. Number of growth units/axis necessary to flowering in function of the order, the treatment and the frequency of fertirrigation.

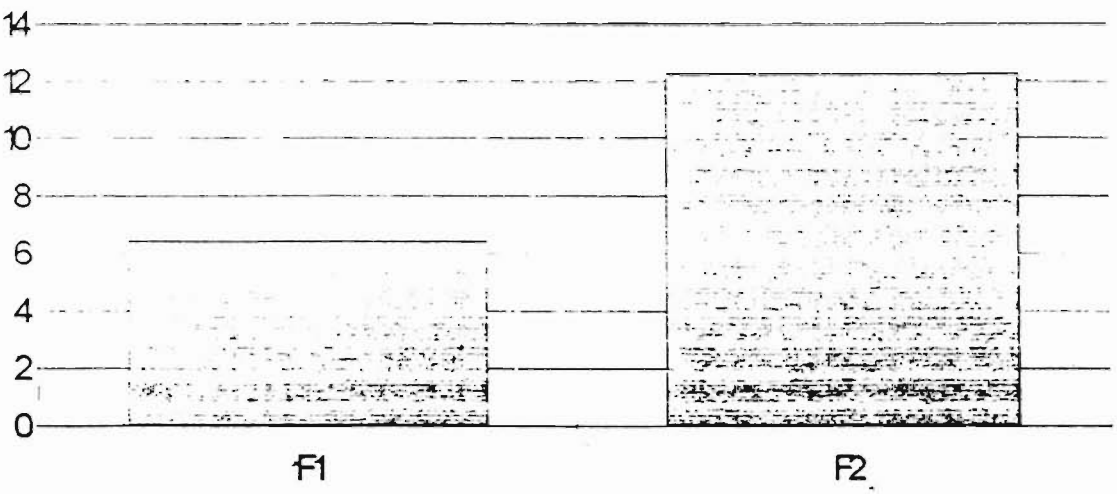

Figure 4. Comparison of the total number of flowers obtained/plant as a function of the fertirrigation frequencies.

The results obtained with *P. neriifolia* seem to confirm those obtained with *P. eximia*, namely the decrease in the number of ramification and GU necessary, for flowering on a given axis. The new element is the improvement of floriferousness brought about by the increase in the watering frequencies. This can be explained by

more regular availability of water and nutrients.

4. Discussion

The experiments conducted on *P. eximia* and *P. neriifolia* show that these two species react the same way to soilless cultivation and to the treatments.

The mode of branching and flowering follows the same principles. These parameters are not modified by growth in soilless conditions. Their potential are increased compared to those of field conditions (Dupee *et al.*, 1992).

The fact that the number of ramifications produced on a given axis decreases with the order of this axis, and that for the first orders, this number increases with the age and perhaps the reserve nutrients of the plant, should be taken into account when pruning the plants. It will be necessary to consider these factors, apparently contradictory, in order to obtain the highest number of flowering stems possible.

In order to produce a flower, there must be a decrease in the number of GU with the order of the axis. Indeed, the further away we are from the central axis, the smaller the number of GU of the flowering stem is. Gradually, the market quality of the product decreases. If it is possible to increase the length of the growth unit, and by doing so, the length of the flowering stem by increasing the frequencies of fertigation, this increase may however remain limited. It is, therefore, necessary to prune back the plants in order to keep an adequate number of GU. The reiteration, or rebuilding of the tree on itself, which could be observed on a free form, could be induced by severe pruning.

Finally, it is interesting to note that to increase the number of flowering shoots, relative to the number of the vegetative axes (in order to increase the productivity of the plant) it may be risky to have all the axes in flower because their harvest can lead to the death of the plant, as we have sometimes noticed in our studies. Therefore, it is necessary to keep some axes in a vegetative state. Those will be used as a permanent vegetative base after the harvest of flowers.

These results do not generally include the seasonal vegetative variations. If these variations are taken into account in a more complete study, we may obtain a better understanding of some of the phenomena, especially those involving flowering.

These results and observations obtained from plants grown in soilless conditions might be extrapolated to growth in the field conditions since the architectural structure of the plant does not depend on the cultivation system, but is a function of the genetic characteristic of the species.

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GROWING PROTEACEAE SOILLESS UNDER SHELTER

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Abstract

Introducing soilless techniques to Proteaceae cultivation, requires the definition of proper nutrient solutions and finding adequate substrates for a plant which has very distinct vegetative cycles. During this preliminary stage, the following questions had to be solved:

- relative N.P.K concentrations and K/(Ca + Mg) ratio at equilibrium conditions;
- chemical compatibility of the substrate in respect of excretions from proteoid roots, these being acidic can attack natural substrates and modify the medium in and around the rhizosphere:
- to maintain the proper growth cycle of the plant, do the vegetative and floral stages imply different water and mineral requirements?

The first question was answered for various species suitable for cut flower production, after successive trials derived from open field experience. The subsequent questions were only considered for *Protea eximia* and *P. neriifolia*. This study enabled us to establish a basic nutrient solution and to list suitable substrates for most Proteaceae genera and species, but relative needs for various development stages remain specific to each species.

1. Introduction

1.1. Vegetative material and environment

Proteaceae culture is intended to provide new incentives to French mediterranean producers who want to diversify their ornamental production. This botanical family, originated from the southern hemisphere (mainly Australia and South Africa), is not very well known in France. It raises considerable interest from its aesthetic point of view and for the variety of colours of its flowers.

Most South African Proteaceae grow on sandy or sandy-clay soils which are well-drained, nutrient deficient and acidic (pH from 4 to 6). These plants have low mineral requirements and are therefore not tolerant of salt concentrations that would appear normal to other plants. As a consequence, the border line between deficiency and toxicity is remarkably narrow in terms of nutrition, and soilless techniques are the only alternative in mediterranean regions, where natural soils are not suitable.

1.2. Soilless culture

Soilless techniques, whatever the species, involve three elements: the container, the substrate and the nutrient solution. Thus, developing a solution for the specific needs of a plant must take into account the chemical nature of the substrate. The container must be watertight and chemically inert, its shape and dimensions depend on the nature of the substrate and on the expected volume of the plant.

This paper contains two parts. The first part recalls the preliminary results obtained during the process of defining a suitable nutrient solution (Montarone *et al.*, 1994), while the second part reports on the corrections that had to be made to the first composition, before any satisfactory cultural result could be obtained for most of the genera and species of this family.

2. Search for a suitable solution

Several articles underline phosphorus as an essential element, required for the safe growth of plants. Claassens and Folscher (1980) notes that nutritional requirements of Proteaceae grown for cut flowers were poorly defined, but that, on the other hand, the influence of proteoid roots on plant growth and development is particularly important (Vogts, 1982; Lamont, 1983; Brits, 1984). Claassens and Folscher (1980) also reports that phosphorus can reduce, in some circumstances, proteoid root production of *Leucospermum* grown on sand. Ellyard and McIntyre (1978) showed that high phosphorus content is a mortality factor for Banksias in western Australia. Nichols and Beardsell (1979) gave evidence of some phosphorus toxicity symptoms on certain Australian Proteaceae. According to Barrow (1977), a low phosphorus content is necessary in leaves of some species if one is to have optimal photosynthesis. They also proved that a high calcium content can worsen leaf necrosis due to high phosphorous content, while high nitrogen and potassium contents can reduce them (Nichols and Beardsell, 1981). Generally speaking, a high phosphorus content can be harmful to Proteaceae in some growth conditions.

With this in mind, we paid special attention to the relative ratios of N, P, K, the main mineral elements responsible for nutrition equilibrium.

2.1. Material and methods

For each of the three mineral elements, N, P and K, a factorial test involving Fisher blocks with two factors was devised. Each element was varied in three ways and applied to five plant varieties.

1) Plant material

Five *Protea* species: *P. cynaroides, P. eximia, P. lacticolor, P. neriifolia, P. obtusifolia*, derived from seeds originating from South Africa, were used.

2) Growth conditions

Seedlings first grown on chemically inert siliceous sand with a 2-3 mm particle diameter range, in small pots of 8 cm in size, were bedded in 7.5 ℓ containers filled with the same sand and placed in a greenhouse kept above 12 °C. The nutrient solution was supplied by drippers, at a rate which varies according to the season and the vegetative stage of the plant. As ordinary tap water was used, the Ca and Mg contents were 5 me and 1 me per litre respectively and these could not be decreased further. Micro-elements were added by means of a commercial solution at the rate of 200 $\mu g.\ell^{-1}$ for Zn and Mn, 100 $\mu g.\ell^{-1}$ for B, and 50 $\mu g.\ell^{-1}$ for Cu and Mo. Fe was supplied as a chelate at a concentration of 800 $\mu g.\ell^{-1}$. Supplies of N, P, and K were varied depending on the experiment.

3) Observations and analysis of results

The effect of each nutrient solution tested was evaluated in terms of stem elongation

but also by the observation of any symptom that could indicate a disfunctioning of the plant growth. A variance analysis and means difference, were determined and treated according to the Newman and Keulz test.

2.2. Results

Five successive tests were enough to obtain an accurate estimate of the required phosphorous content, the N.NH4/[N.NH4 + N.NO3], P/N, K/N ratios and finally, the total salt concentration. Only the results of the last trial concerning potassium is presented. Table 1 indicates the three experimental nutrient solutions. The resulting stem length increases for five species of Proteaceae are shown in table 2.

Table 1. Test nutrient solutions with three different compositions.

	Solution 1					Solution 2			
S1(me)	NO_3^-	$PO_4H_2^-$	$SO_4^=$	Σ	S2(me)	NO_3^-	$PO_4H_2^-$	$SO_4^=$	Σ
K^+	0.250			0.250	K^+	0.313		0.189	0.502
NH_4^+	0.188	0.125	0.188	0.500	NH_4^+		0.125	0.188	0.313
H^+					H^+				
Σ	0.438	0.125	0.188	0.750	Σ	0.313	0.125	0.377	0.815

Solution 3				
S3	NO_3^-	$PO_4H_2^-$	$SO_4^=$	Σ
K^+	0.313		0.688	1.001
NH_4^+		0.125	0.188	0.313
H^+				
Σ	0.313	0.125	0.876	1.314

Table 2. Length increase (cm), for species of *Proteaceae*.

	Species				
Treatments	*P. cynaroides*	*P. eximia*	*P. lacticolor*	*P. neriifolia*	*P. obtusifolia*
solution 1	13.37 (a)	2.49 (a)	5.18 (a)	5.33 (a)	14.93 (ab)
solution 2	11.0 (a)	3.69 (a)	25.71 (bc)	15.96 (a)	15.53 (ab)
solution 3	7.48 (a)	1.38 (a)	32 (c)	3.69 (a)	10.03 (a)

Mean values carrying the same letter are not significantly different (P = 0.05).
Interaction (species x treatments) : F value = 8.06 F (P = 0.01) = 3.36.

The analysis of variance of these results shows a distinct behaviour for *P. lacticolor* compared to other species. Solutions S2 and S3 gave the best results for this species, whereas they gave rather poor results on others. It could be concluded that S2, the median solution, is the best compromise as a common solution suitable for most species.

2.3. Conclusion

This study enabled us to make considerable progress defining a nutrient solution suitable for growing Proteaceae without soil. A general fertilization ratio emerges from the whole set of observations, with N, P, and K contents being proportional to 1 — 0, 1 — 0.7. $N.NH_4:NO_3$ ratio for nitrogen was found to be 1:1 and the total salt content amounts to 0.8 me per litre.

3. Applications to Protea cultivation

As this first attempt appeared satisfactory, a collection of 20 species was put under soilless culture conditions. In addition, two extra experiments were undertaken to investigate the ramification modes (branching patterns) of *P . eximia* and *P. neriifolia* with porphyry from the Esterel (rhyolitic rock with alkaline feldspar) as a substrate.

Cultural problems encountered with *P. eximia* contributed to finding a better estimate of the composition of the appropriate nutrient solution. Moreover, the experiment carried out on *P. neriifolia* underlined the incidence of mineral inputs on the development of the plant and the variation of the requirements according to the growth stage.

3.1. Material and method

3.1.1. *P. eximia* trial

Plants were obtained from seed beds consisting of 40% siliceous sand and 60% sieved peat. They were bedded in porphyry from the Esterel, a local and cheap substrate with a granule size of 5 to 8 mm.

The experimental set-up comprised 36 polypropylene benches of dimensions, 3.00 x 0.90 x 0.18 m; there were 2.2 plants.m^{-2}. Fertigation was supplied by drippers. Each plot received a minimum of three ℓ of nutrient solution per day, inputs being increased as plants developed and according to the season. The statistical layout involved 12 Fisher blocks.

3.1.2. *P. neriifolia* trial

Plants were obtained from seed beds on the same medium as above, and placed individually in 55 ℓ containers filled with Esterel porphyry of the same granule size as for *P. eximia*. The experimental setup, comprising 64 containers with one plant in each, was divided into eight Fisher blocks. Fertigation was supplied with fixed time intervals by drippers. Two treatments with distinct time intervals were applied in order to assess the influence of water and mineral element supply status. At the lower frequency rate (F1), a mean value of three ℓ of nutrient solution was delivered in a day, at the higher frequency rate (F2) the mean delivered volume was doubled. These inputs were varied with the vegetative stage and the season.

Greenhouse climate conditions were similar in the two trials, winter heating at +5 °C and day ventilation at +20 °C.

The composition of the nutrient solution, expressed in milli-equivalents, is shown in table 3.

Table 3. Nutrient solution composition for *P. eximia* and *P. neriifolia*.

me.ℓ^{-1}	NO_3^-	$PO_4H_2^-$	$SO_4^=$	Σ
K^+	0.313		0.189	0.502
NH_4^+		0.125	0.188	0.313
H^+				
Σ	0.313	0.125	0.377	0.815

Trace elements: Fe, 800 $\mu g.\ell^{-1}$, Zn and Mn, 200 $\mu g.\ell^{-1}$, B, 100 $\mu g.\ell^{-1}$, Cu and Mo, 50 $\mu g.\ell^{-1}$.
Tap water accounted for 7 me calcium, and 3 me magnesium.

3.2. Results

As a whole, plants from all species managed to produce a satisfactory growth during the early 18 months of the trials; some disfunction did appear under various conditions, but the detailed procedures for analysing and solving such problems were only undertaken for *P. eximia*, which was being studied within a simpler experimental setup and were then applied to *P. neriifolia*.

3.2.1. *P. eximia* trial

This species displayed strong chlorosis symptoms, as well as leaf reddening, which could lead to complete drying of the plant. Most treatments designed to correct these effects were useless, except the increase of irrigation frequency. The trial was carried out as follows:
- ⅓ of the containers received 65 $\ell.day^{-1}$ of solution;
- ⅓ of the containers received 104 $\ell.day^{-1}$;
- ⅓ of the containers received 143 $\ell.day^{-1}$.

The percentage of plants which did not show chlorosis were observed as a function of applied volume (figure 1). It clearly indicates that the incidence decreases when the irrigation volume increases.

In order to explain this observation, substrate samples were extracted, either in the vicinity of the roots, or away from roots, in between two plants (table 4). This table indicates a very high salinity level in the whole medium, and underlines the higher salt concentrations in regions close to the roots, compared with regions away from the roots. Such high concentrations are most probably detrimental to the plants.

Table 4. Substrate analysis.

	Total conductivity	Total soluble salts	Soluble salts without SO_4^{--}	N: NO_3^-	N: NH_4^+	P	K	Ca	Mg
	mS.cm^{-1} 20°C	g.ℓ^{-1}	g.ℓ^{-1}	g.ℓ^{-1}	mg.ℓ^{-1}	mg.ℓ^{-1}	g.ℓ^{-1}	g.ℓ^{-1}	g.ℓ^{-1}
Around roots	128.5	119.5	16.6	1.0	18.0	21.8	1.24	24.4	2.8
Away	93.8	87.2	13.3	0.6	16.1	10.9	0.8	16.0	2.0

Considering observations on the behaviour of proteoid roots (Gardner *et al.*, 1981), one can propose some explanation of the present symptoms. If proteoid roots can strongly acidify their immediate surrounding, porphyry, a rhyolitic rock containing 65% alkaline feldspar, can undergo chemical decay in such regions and release several kinds of minerals that alter the composition of the local solution. A subsequent rise of pH will induce the precipitation of most metal cations and induce deficiencies and toxicity.

Given the very high salt concentrations observed in the medium, plants were rinsed with pure water during a few weeks, until a deficiency was detected of N, P, and K in the leachates. The leachate composition enabled us to decide when to switch back to the nutrient solution. Table 5, which exhibits variable results during several successive weeks, indicates how difficult it was to get back to the correct mineral balance, in the presence of a root system which apparently acidifies the medium.

Table 5. Leachate analysis (from December 1991 to July 1992).

Year/month	pH	E.C.	NO_3^-	$PO_4H_2^-$	NH_4^+	K^+	Ca^{++}	Mg^{++}
		$\mu S.cm^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$
Initial solution	6.9	857	0.32	0.045	0.25	0.31	5.80	3.46
91/12	8.1	815	0.56	0.03	0	0.30	5.8	3.1
92/01/10	8.2	793	0.58	0.04	0	0.30	5.9	3.1
92/01/25	7.8	849	0.70	0.05	0	0.31	6.1	3.5
92/02/04	7.9	946	0.70	0.05	0	0.38	6.2	4.0
92/02/18	7.6	828	0.50	0.05	0	0.37	6.10	2.9
92/03/03	7.3	818	0.43	0.04	0	0.37	6.0	2.5
Deionized water	5.4	10	0.02	0	0	0.0	0.11	0.04
92/03/18	7.4	120	0.03	0.04	0	0.11	0.77	0.29
92/03/31	7.5	510	0.29	0.03	0	0.24	3.60	1.39
92/04/13	7.7	410	0.26	0.02	0	0.17	2.92	1.07
Deionized water + N + K	6.8	150	0.29	0	0.34	0.39	0.10	0.05
92/04/29	7.3	190	0.32	0.02	0	0.24	1.31	0.36
92/05/12	7.8	179	0.26	0.02	0	0.30	0.95	0.28
92/06/02	6.9	252	0.46	0.04	0	0.45	1.18	0.35
92/06/23	6.6	127	0.26	0.03	0	0.32	0.54	0.14
Nutrient solution	4.9	247	0.41	0.07	0.32	0.49	0.62	0.30
92/07/10	6.4	270	0.31	0.03	0	0.51	1.17	0.31

Table 6 reports leachate analysis from January to May 1993. As these results appear satisfactory and relatively homogeneous, it is deduced that the resulting composition of nutrient solution is acceptable for soilless cultivation of Proteaceae.

Table 6. Leachate analysis (from January 1993 to June 1993).

Year/month	pH	EC	NO_3^-	$PO_4H_2^-$	NH_4^+	K^+	Ca^{++}	Mg^{++}
		$\mu S.cm^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$	$me.\ell^{-1}$
Nutrient solution	4.7	235	0.46	0.0 7	0.3 4	0.46	0.52	0.32
93/01/11	6.08	184	0.48	0.02	0	0.27	0.80	0.29
93/03/15	6.60	248	0.46	0.02	0	0.28	0.87	0.36
93/03/30	5.90	271	0.42	0.03	0	0.51	0.84	0.38
93/04/16	6.03	235	0.37	0.02	0	0.37	0.94	0.38
93/04/30	6.61	254	0.35	0.02	0	0.29	0.74	0.30
93/05/15	6.20	229	0.34	0.02	0	0.35	0.87	0.35
93/05/30	7.00	342	0.41	0.03	0	0.29	0.50	0.20

3.2.2. *P. neriifolia* trial

This trial was run with leachate analysis at constant intervals, with a solution initially modified according to that of *P. eximia*. The results are shown in table 7.

Table 7. Leachate composition for *P. neriifolia* according to the season (me.ℓ^{-1}).

		pH	μS^{-1}.cm	N: NO_3^-	$PO_4H_2^-$	N: NH_4^+	K^+	Ca^{++}	Mg^{++}
	Input sol.	6.6	876	0.43	0.07	0.34	0.43	5.76	3.82
Winter**	freq. 1*	7.8	861	0.5	0.06	0	0.36	6.44	3.41
	freq. 2*	7.5	859	0.52	0.06	0	0.42	6.29	3.58
	Input sol.	4.2	296	0.51	0.08	0.34	0.47	0	0
Spring**	freq. 1	6.9	397	0.22	0.02	0	0.33	2.0	0.74
	freq. 2	5.8	299	0.36	0.03	0	0.31	1.20	0.54
	Input sol.	4.4	195	0.41	0.07	0.31	0.46	0	0
Summer	freq. 1	6.7	340	0.06	0.03	0.	0.48	2.02	0.67
	freq. 2	6.6	212	0.34	0.03	0	0.40	0.90	0.28
	Input sol.	4.8	248	0.61	0.07	0.37	0.47	0.63	0.34
Autumn	freq. 1	7.7	359	0.52	0.03	0	0.40	1.35	0.55
	freq. 2	7.5	330	0.64	0.04	0	0.39	0.98	0.46

*freq. 1 = leachate values corresponding to low frequency irrigation F1

*freq. 2 = leachate values corresponding to high frequency irrigation F2

** during winter the nutrient solution was prepared with tap water which explains the pH value and the high observed contents in Ca and Mg; at spring time the substrate was rinsed with pure (deionized) water, and the solution used later on was then free from Ca and Mg.

Even if the present nutrient solution is not yet perfectly suited to actual plant requirements, this table shows firstly, distinct leachate compositions depending on the irrigation frequency and secondly, definited periods during which absorption becomes more important. Figure 2 stresses the observed fluctuations according to the development period.

In winter, whatever the irrigation frequency, plants absorb 85% of the applied nitrogen and 80% of the applied potassium. In spring, the figure for N rose to 94% for the (low irrigation) F1 frequency and 90% for the (high irrigation) F2 frequency. The figures for K were respectively 84% and 85% for F1 and F2. In summer, the leachates are completely depleted of nitrogen for the F1 and 90% depleted for F2, while figures for K are 75% and 81%. During autumn, N and K absorption rates returned to the winter values.

Calcium and magnesium were more abundant in the leachates than in the applied solution : the increase is 56% for F1 and 20% for F2. When the plants were supposedly at rest, this increase was still 36% for F1 and 23% for F2.

From these various trials, it was possible to deduce a suitable composition of nutrient solution at least in terms of its major elements (table 8).

Table 8. Composition of the nutrient solution (me.ℓ^{-1}).

	NO_3^-	$PO_4H^=$	$SO_4^=$	Cl^-	Σ
K^+	0.43				0.43
NH_4^+		0.07	0.27		0.34
Ca^{++}				0.70	0.70
Mg^{++}				0.30	0.30
Σ	0.43	0.07	0.27	1.00	1.77

Micro-elements could not yet be investigated, but the ordinary blend from the market seems to suit plant requirements, provided some additional Fe is added in the form of chelate.

Leachate analysis strongly indicated two distinct periods for plant nutritional demand. These periods vary with the respective species.

4. General conclusions

The original objective of the work was reached after a series of trials:
- a mineral composition was determined which suits most Proteaceae grown soilless under shelter, as testified by leachates eventually stabilizing in composition.

Some questions remain:
- why is there such a high calcium excess in the leachates at bud break? This could be due to some chemical reaction induced in the porphyry substrate. It seems therefore advisable to use the chemically most inert medium for such plants.

Some interesting observations include:
- comparing the effect of two irrigation frequencies (and thus two irrigation volumes) can be interpreted in two different ways. First, increasing the leachate rate is an obvious way of correcting salinity drifts in the medium, it allows to displace and renew the mineral content of the medium at a higher rate, and reduces the risk of toxicity, especially when roots acidify the substrate and release some of its compounds. On the other hand, it also avoids local depletion of some minerals in the medium, when the plant demand exceeds the nutrient availability, this particularly being the case during periods of fast growth, like summer. Both effects can contribute to modify the yield and the quality of the crop, from treatment F1 to treatment F2, as presented in table 9.

Table 9. Flower quality according to irrigation frequency (plants were two years old).

	Mean number of flowers.plant^{-1}	Mean length of flower stems (cm)
F1	6	58.12
F2	11	72.17

Phosphorus absorption measurements could not be performed because of alkalinization of the medium. Solid deposits occuring inside the medium would have distorted observed values in the leachates, as this was already noticed (Salaün, 1990).

Despite several useful findings, the trials described here were not able to solve the complete list of problems that can be encountered when trying to establish efficient soilless culture. It is necessary, in particular, to know more about the precise mineral and water requirements of the plant for its various vegetative stages, which can only be done by using a chemically inert medium.

P. neriifolia was responsible for a marked dilution of N and K in the leachate, when flower buds appeared. According to Hanekom *et al.* (1973), such a faster absorption could come from some increased activity of proteoid roots at that time. This is a topic which deserves more attention, since most authors only quote those roots as having some chemical activity. What about the other part of the root system? In soilless cultures, and for most species, including Proteaceae, the root system is particularly well developed, but its morphology is quite different from a system developing in deep soil. Is water and mineral absorption only correlated to the amount of proteoid roots? Some authors believe that proteoid roots could be less developed in the case of optimum feeding conditions (water and nutrients). Lamont (1983), on the other hand, states that such roots develop under favourable conditions as well, and contribute to increase the plant yield.

Clearly improved knowledge on the role of proteoid roots in plant development is needed to further improve soilless growing techniques on Proteaceae.

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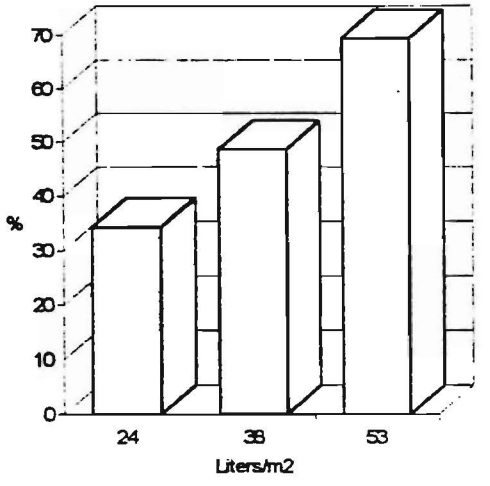

Figure 1. Percentage of non-chlorific plants according to fertigation.

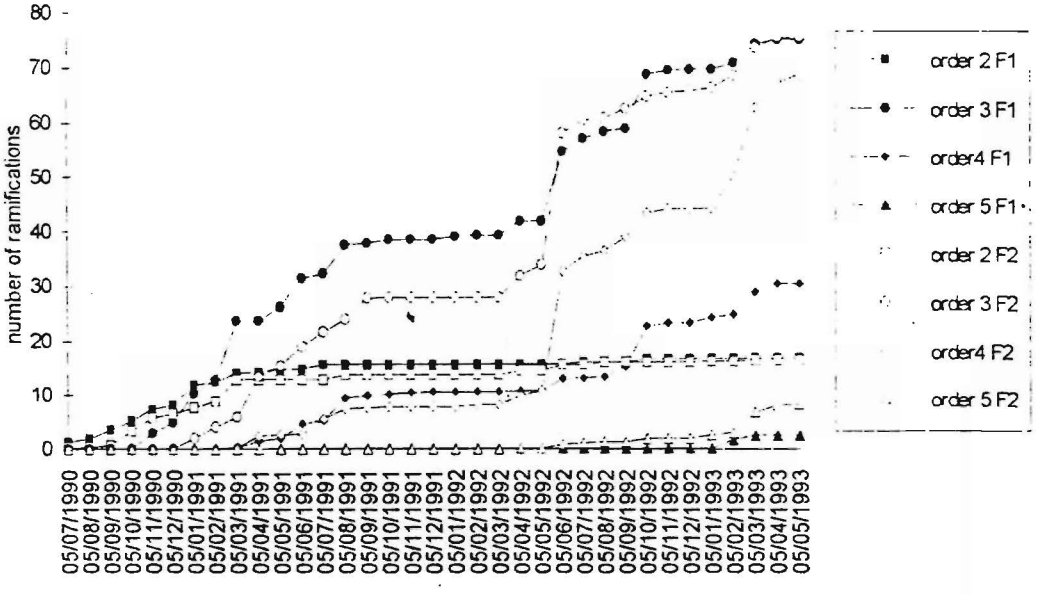

Figure 2. Plant development according to the year and the frequencies.

EFFECTS OF TREATMENT WITH GIBBERELLIC ACID ON GERMINATION OF PROTEA CYNAROIDES, *P. EXIMIA, P. NERIIFOLIA* AND *P. REPENS* (PROTEACEAE)

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Abstract

The seeds of South African *Protea* spp. (botanically achenes) show dormancy, which seems to be imposed by a low temperature requirement and by the action of the pericarp.

In order to study the effects of gibberellic acid (GA_3) on germination of *Protea cynaroides*, *P. eximia*, *P. neriifolia* and *P. repens*, seeds of those species were sown in a mixture of peat moss and lapilli (1:1 in volume), in plastic propagating trays which were placed in a well ventilated greenhouse. Before sowing, seeds were imbibed for 24 h in either GA_3 (100 ppm) or distilled water (control). A randomized block design with two treatments and three replications was employed. Fifty seeds per treatment were used. The total number of seeds was three hundred for each species. Germination was recorded weekly, for 21 weeks. Percentages and rates of germination were calculated.

Imbibition by *P. eximia* and *P. neriifolia* seeds of a GA_3 solution (100 ppm) for 24 h, improved significantly its percentage and rate of germination. Although GA_3 treatment improved percentage and rate of germination of *P. cynaroides*, this effect was not statistically significant. *P. repens* germination percentages were abnormally low and were not taken into account.

1. Introduction

The seeds of South African *Protea* spp. (Proteaceae), botanically achenes, show dormancy. Germination takes place over extended periods of time and germination percentages are usually low. Scarification, stratification and incubation in pure oxygen have improved the germination of *P. compacta* (Brown and Van Staden, 1973).

Dormancy seems to be imposed by a low temperature requirement and by the action of the pericarp, which prevent simultaneous germination of all seeds (Deall and Brown, 1981). Brits (1986) suggested that in proteaceous serotinous species (e.g. *Protea*) there is only a low incubation temperature requirement but no need during germination for an increase in the partial pressure of oxygen.

It is known that the use of gibberellins can often substitute for the low temperature requirement (Taylorson and Hendricks, 1977). However, treatment with GA_3 depressed the germination of *P. compacta* seeds (Mitchell *et al.*, 1986).

In this study, the effect of GA_3 treatment on germination of *P. cynaroides, P. eximia, P. neriifolia* and *P. repens* seeds was investigated. These species are being grown in Tenerife and their cut flowers exported to Europe.

2. Material and methods

Seeds of *P. cynaroides*, *P. eximia*, *P. neriifolia* and *P. repens* were used. These were obtained from the National Botanic Gardens, Kirstenbosch, South Africa.

After disinfection in hot water at 50 °C for 30 minutes, seeds were imbibed for 24 h in either distilled water (control) or a 100 ppm GA_3 solution. Then, they were dried and treated with thiram powder (80% a.i.). Seeds were sown in a mixture of lapilli and peat moss (1:1 v/v) in plastic propagating trays which were placed in a well ventilated greenhouse. After sowing, seeds received a drench of a 2 $g.\ell^{-1}$ benomil (50% a.i.) solution and were irrigated weekly with water containning 2 $g.\ell^{-1}$ of benomil (50% a.i.) and captab (50% a.i.), alternatively.

A randomized block design with two treatments and three replications was employed. Fifty seeds for each treatment were used.

Newly germinated seeds were counted weekly for 21 weeks after sowing. Percentages and rates of germination were calculated, the latter by means of the formula of Maguire (1962). The results were subjected to analysis of variance and to the Duncan test. The trial was carried out between January and June, 1987, in the Jardín de Aclimatación de La Orotava, Puerto de la Cruz (Tenerife).

3. Results and discussion

3.1. *P. cynaroides*

Seeds of treatment 2 (100 ppm of GA_3) began to germinate 22 days after sowing, 5 days before those of treatment 1 (control) (figure 1). Although the final germination percentage of the former treatment (60.7%) was higher than the latter (52%), the difference at 5% level was not significant. The germination rates were 0.873 and 0.613, respectively, also with no significant difference between the GA_3 treatment and the control.

Final germination percentages were higher than those given by Parvin *et al.* (1973) (44%), Van Staden (1966, cited by Van Staden and Brown, 1977) (31%) and Brits (1986) (35.7%) and lower than the figure given by Horn (1962, cited by Van Staden and Brown, 1977) (66%).

The results obtained did not agree with the hypothesis that the GA_3 treatment could improve germination. Although an improvement in percentage and rate of germination was observed, these were not statistically significant.

3.2. *P. eximia*

Seeds of both treatments (GA_3 and control) germinated at the same time as those of *P. cynaroides*, at 22 and 27 days, respectively. The GA_3 treatment gave the highest percentage (44.7%) and rate (0.647) of germination, which were significantly greater than the control (14% and 0.187), at the 5% level (figure 2).

The germination percentage of the GA_3 treatment was between the values given by Van Staden (1966, cited by Van Staden and Brown, 1977) (50%) and Parvin *et al.* (1973) (40%).

These results showed that the GA_3 treatment improved germination significantly, in agreement with results given by Brown *et al.* (1986) for *Leucospermum cordifolium*, a South African proteaceous species with nut-like seeds

also showing dormancy. However, in *P. compacta*, the GA_3 treatment depressed germination (Mitchell *et al.*, 1986).

3.3. *P. neriifolia*

Seeds of treatment 1 (control) began to germinate 29 days after sowing, 7 days later than those of treatment 2 (100 ppm GA_3) (figure 3). Seeds of this species showed the same germination pattern than those of *P. eximia*. The GA_3 treatment gave the highest percentage (52.7%) and rate (0.750) of germination. These values were significantly different from those of the control (10.7% and 0.123), at the 5% level.

The germination percentage of the GA_3 treatment was lower than that given by Horn (1962, cited by Van Staden and Brown, 1977) (66%) and slightly higher than that given by Parvin *et al.* (1973) (48%).As in *P. eximia*, germination was significantly improved by the action of GA_3.

3.4. *P. repens*

Seeds of both treatments began to germinate 26 days after sowing. The final percentages and rates of germination were very low, being 16.7% and 0.20 for the control and 17.3% and 0.17 for the GA_3 treatment.

As these germination percentages were much lower than those given by Horn (1962, cited by Van Staden and Brown, 1977) (33%) and by Brits (1986) (24%), perhaps because of the low viability of seeds, these results were not taken into account.

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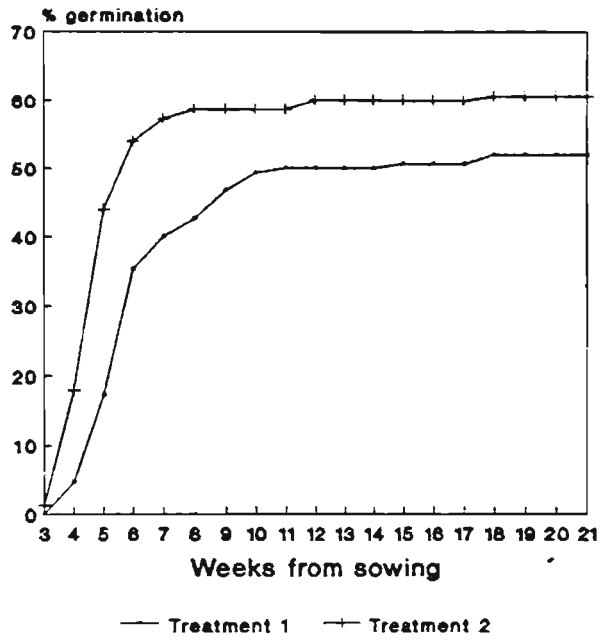

Figure 1. GA_3 effects on germination of *Protea cynaroides*. Treatment 1 = control; Treatment 2 = GA_3 (100 ppm.)

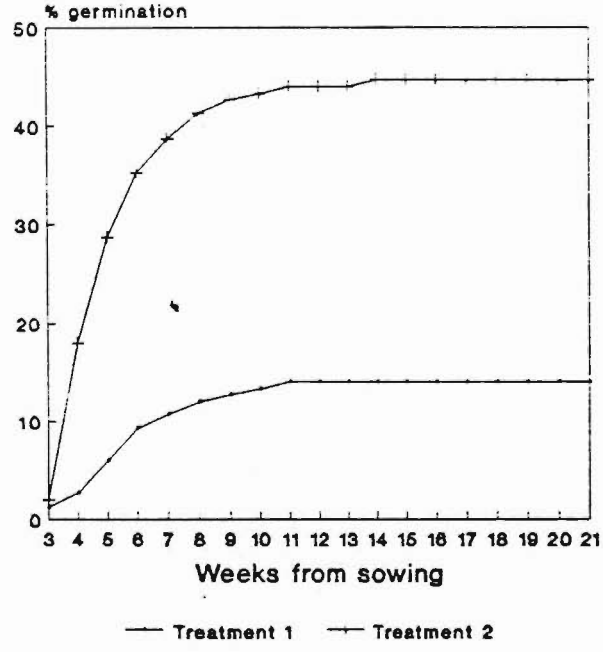

Figure 2. GA_3 effects on germination of *Protea eximia*. Treatment 1 = control; Treatment 2 = GA_3 (100 ppm.)

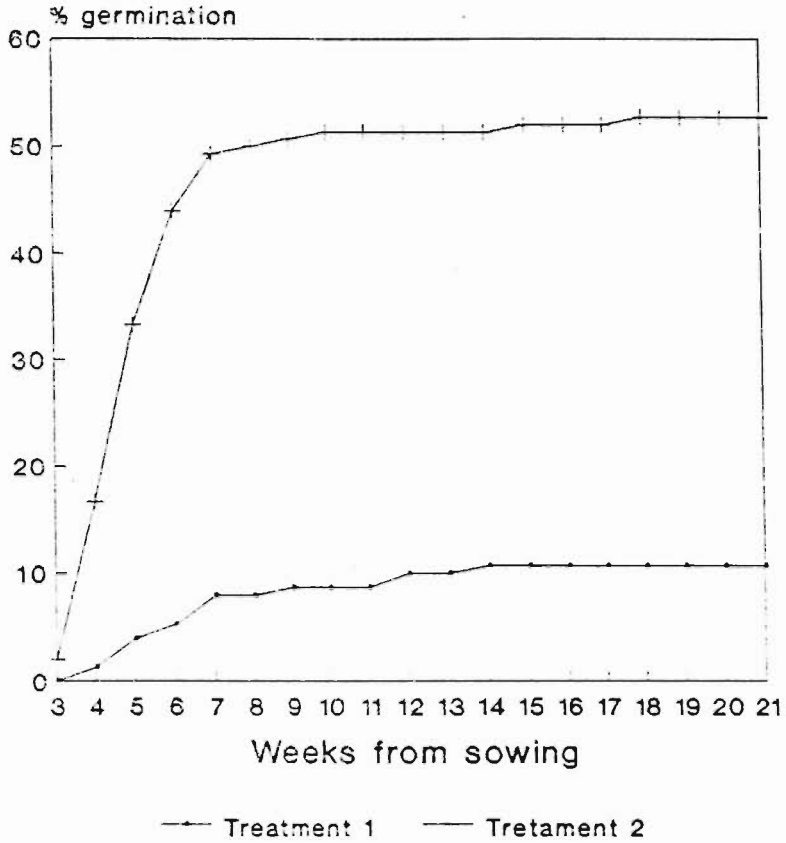

Figure 3. GA_3 effects on germination of *Protea nerifolia*. Treatment 1 = control; Treatment 2 = GA_3 (100 ppm)

PRELIMINARY INVESTIGATION INTO THE EFFECT OF TIME OF PRUNING ON SHOOT GROWTH AND FLOWERING TIME OF *PROTEA*

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Abstract

Shoot growth, flower initiation and development cycles of *Protea* are not as predictable as in *Leucospermum* and *Leucadendron*. Flower initiation apparently does not occur at the same time in different genotypes. This very often inhibits consistent predictable production of high quality long stemmed *Protea* cut flowers. This study reports on attempts to regulate these processes through pruning.

Shoots of *Protea* cultivars Cardinal, Brenda, Sneyd and Susara were pruned during June, September, December and March. Mature shoots were cut back to the bud ring separating the first and second flush of the shoot to leave a stump of 10 to 15 cm on the plant. The growth of the strongest shoot, the increase in diameter of flowers on this shoot and its flowering time were monitored monthly. Implications for cutting plant training and pruning of *Protea* are discussed.

1. Introduction

Proteas are grown commercially as cut flowers in South Africa, mainly for export to European flower markets. Approximately 20 species and 10 cultivars are currently marketed representing approximately 12 types of products (product lines). Shoot growth flushes are produced from spring to autumn. Inflorescence development, between and often within types, can seemingly commence over extended periods. Flower harvesting and pruning of proteas are traditionally synonymous (Brits *et al.*, 1986), mainly due to seedling derived orchards making predictable reaction to standardized pruning practices almost impossible. Pruning standards for the short day plants *Leucospermum* and *Leucadendron* were quickly established and can be implemented and adapted to new selections with relative ease. The *Protea* inflorescence is borne terminally on a shoot which consists of 2 to 5 growth flushes. Flushes arise in succession from a distal axillary bud. Flushes usually exhibit strong apical dominance during active growth. However in some species/cultivars brancing occurs distally on flushes at the intercalation (transitional area between flushes). The flowering time of species vary within and between types to the extent that the collective marketing period of the product lines vary from 2 months (*P. pityphylla*) to year round production (*P. cynaroides*).

No research has been done on the influence of pruning on the flowering time of *Protea*. In this study we investigated the effect of pruning at different times of the year on subsequent shoot (flower stem) and flower development and on the time of inflorescence opening.

2. Methods and Material

2.1. Plant material

Plants of *Protea* cultivars 'Cardinal' (*P. eximia* x *P. susannae* x *P. eximia*), 'Brenda' (*P. compacta* x *P. burchelli*), 'Sneyd' (*P. repens*) and 'Susara' (*P. susannae* x *P. magnifica*) in a cultivar maintenance plantation were used in this research. The plants were 4 years old and had previously been propagated from the same clone. They were grown under natural climatic conditions at Elsenburg, Cape Province, South Africa (33°54'S). Plants were spaced 2 m apart with 4 m between rows, clean cultivated, and fertilized with 40 g ammonium sulphate per plant during spring and autumn. Annual rainfall for the area is 600 to 700 mm and occurs mainly during winter. Plants were irrigated 128 ℓ.week^{-1} during the dry summer months.

2.2. Pruning

Fully mature shoots were pruned to the intercalation separating the bottom two flushes of the shoot on 10 June, 12 September, 12 December and 12 March. Twenty five shoots were randomly selected from 25 plants on every date. The length of the strongest axillary shoot, which developed on the stump, was measured monthly. The development of the inflorescence on this strongest shoot was monitored by measuring its diameter monthly from its appearance to anthesis.

3. Results and Discussion

3.1. Shoot growth

Shoots developed from upper axillary buds on shoots cut back on all dates. The time from cutting back to sprouting of the buds were related to the time of pruning (figure 1). Buds on shoots pruned during June initiated growth during August to September (figure 1). September and December pruning resulted in immediate sprouting in all varieties. March pruning resulted in an immediate short autumn flush in Sneyd and Cardinal (figure 1), with Brenda and Susara initiating axillary growth during May and June respectively (figure 1). Subsequent terminal growth flushes on axillary shoots were produced seasonally, until inflorescence initiation. Four indistinct periods of flushing were evident: (1) short winter flushes produced from March to August; (2) long spring flushes produced from September to November; (3) medium to long summer flushes produced from December to January; and (4) medium to long autumn flushes produced from February to March. Shoots of all cultivars which completed a second or further flush outside the natural period for flower initiation, tended to continue vegetative development from distal axillary buds (data not presented). This resulted in undesirable, branched flower stems and where only axillary shoots developed, flower abortion at a later stage.

The vigour of shoots differed considerably, and all the shoots did not flush during each period. This resulted in the shoots not all being ready to flower at the same time. The average flowering shoot lengths were not significantly different, between pruning dates, in Sneyd and Susara (figure 1). In Brenda, March pruning

resulted in a significant decrease in flowering shoot length. Cardinal flowers produced following September pruning were borne on the longest stems (figure 1), but the stem length of all except the March pruning dates were variable, dependent on the number of flushes (2 to 5) produced prior to flower initiation. March pruning resulted in the shortest average Cardinal flowering stems, with most shoots initiating flowers on the second flush.

3.2. Flower initiation and development.

The period during which flower initiation occurred, can be estimated to be some time between cessation of shoot elongation growth and the measurable increase in inflorescence bud diameter. All flowers were initiated on shoots consisting of 2 or more flushes.

Sneyd initiated flowers, between April and August, almost exclusively on autumn flushes. The shortest time from pruning to flower initiation was 5 months following December pruning, with inflorescences requiring approximately 11 months of growth to reach opening.

Brenda and Susara initiated flowers only on spring or summer flushes. In Brenda such shoots were produced within 5 months following June pruning, the inflorescences taking 6 months to reach opening. In Susara flower stem production took at least 11 months, following December pruning, and inflorescence development 6 months.

Cardinal flowers were initiated non-seasnonally, on flushes formed during all seasons. The period from pruning to flower initiation depended on the number of flushes produced prior to initiation. On shoots consisting of 2 flushes, flowers were initiated within 3 months following September pruning and reached opening within 7 months from initiation (figure 2). The erratic behaviour of the lines for Cardinal, representing the average flower diameter, reflects this flower initiation pattern (figure 2).

3.3. Flowering time and percentage

The desired marketing period for proteas in Europe is from August to Mother's day (May), with June and July considered to be poor months for export of cut flowers. This implies that a shift of even one month in flowering time may have significant marketing consequences. Sneyd flowered from February to April with peak flowering during March, irrespective of the pruning date (figure 3). Brenda flowered from March to August with June pruning resulting in late flowering 13 months after pruning and early flowering after 20 months (figure 3). September and December pruning resulted in May to June flowering, and March pruning caused July flowering of Brenda (figure 3). Susara flowered from March to June with undesirable later flowering following December pruning (figure 3).

In these cultivars many flowers aborted on shoots following June and September pruning, resulting in the highest flower percentage following December pruning. Flower abortion occurred, probably due to competition from axillary shoots, which developed prior to flower initiation due to shoot growth cessation outside the natural flower initiation period (data not presented).

4. Conclusion

The span of three to 11 months required for *Protea* flower shoot development, and the seven to 11 months required for flower development, demonstrate the enormous differences in shoot growth and flower developmental patterns within the genus, but which forms the basis of production pruning in *Protea*. The optimal time of pruning for quality flower production, would seem to be the shortest time from pruning to anthesis, approximately as follows:

(1) 16 to 18 months for Sneyd flowering during April i.e., October to December,
(2) 14 to 15 months for Brenda flowering during April i.e., January to February, 14 to 15 months for Brenda flowering during August i.e., May to June,
(3) 17 to 19 months for Susara flowering during April i.e., September to Novomber, Susara flowering during August i.e., December to February,
(4) 9 to 11 months for Cardinal to obtain the required flowering time. This approach to pruning, to obtain optimal quantity of saleable stems, does not allow for production of longer shoots (3 to 4 flushes), and also need to be further refined as the two to three month pruning period will not ensure accurate prediction of harvesting times in most cultivars.

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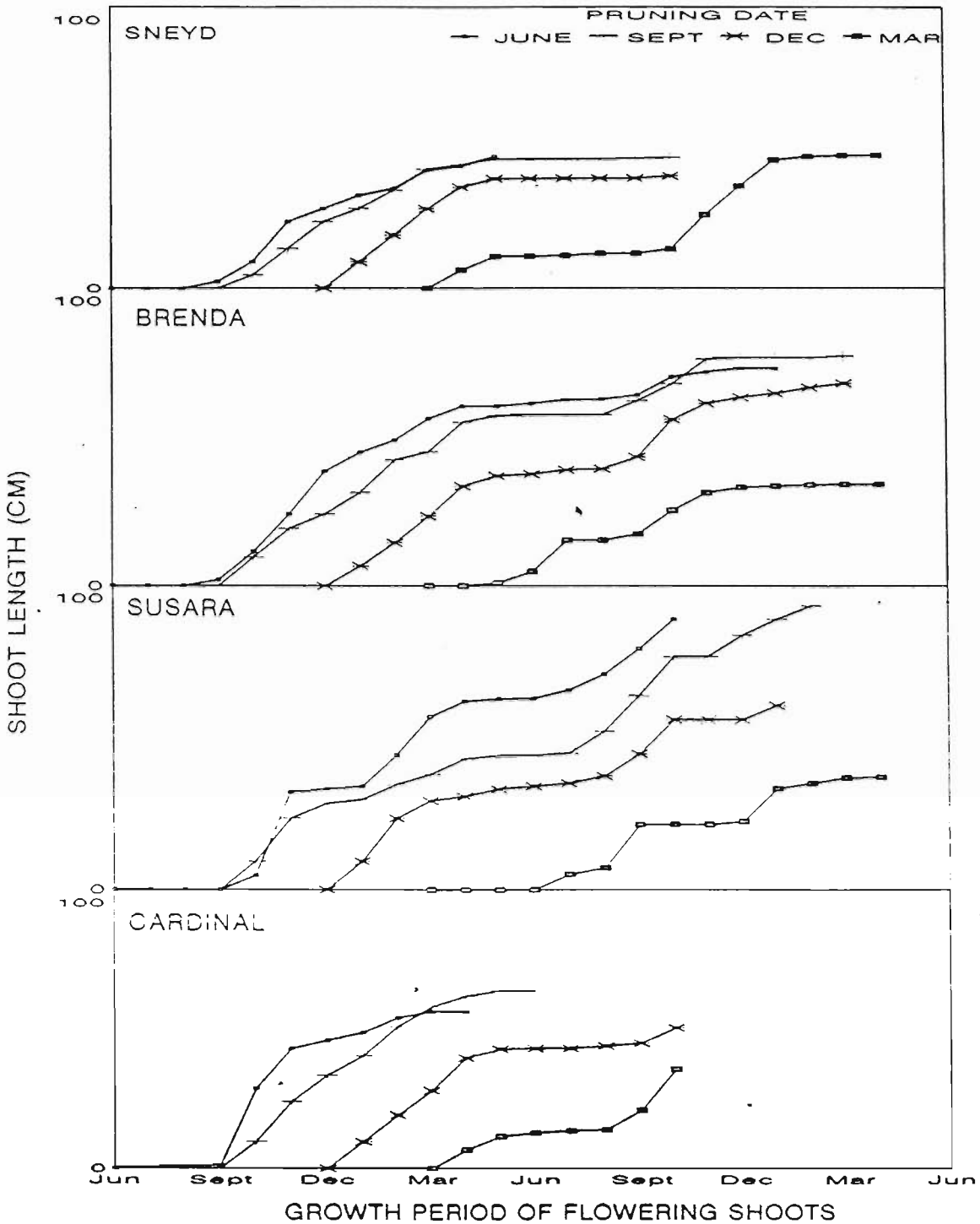

Figure 1. Length of the strongest axillary shoot, measured monthly, of four *Protea* cultivars following pruning on four different dates as indicated. Treatments consisted of 25 single shoot replicates and data represent the length only of flowering shoots.

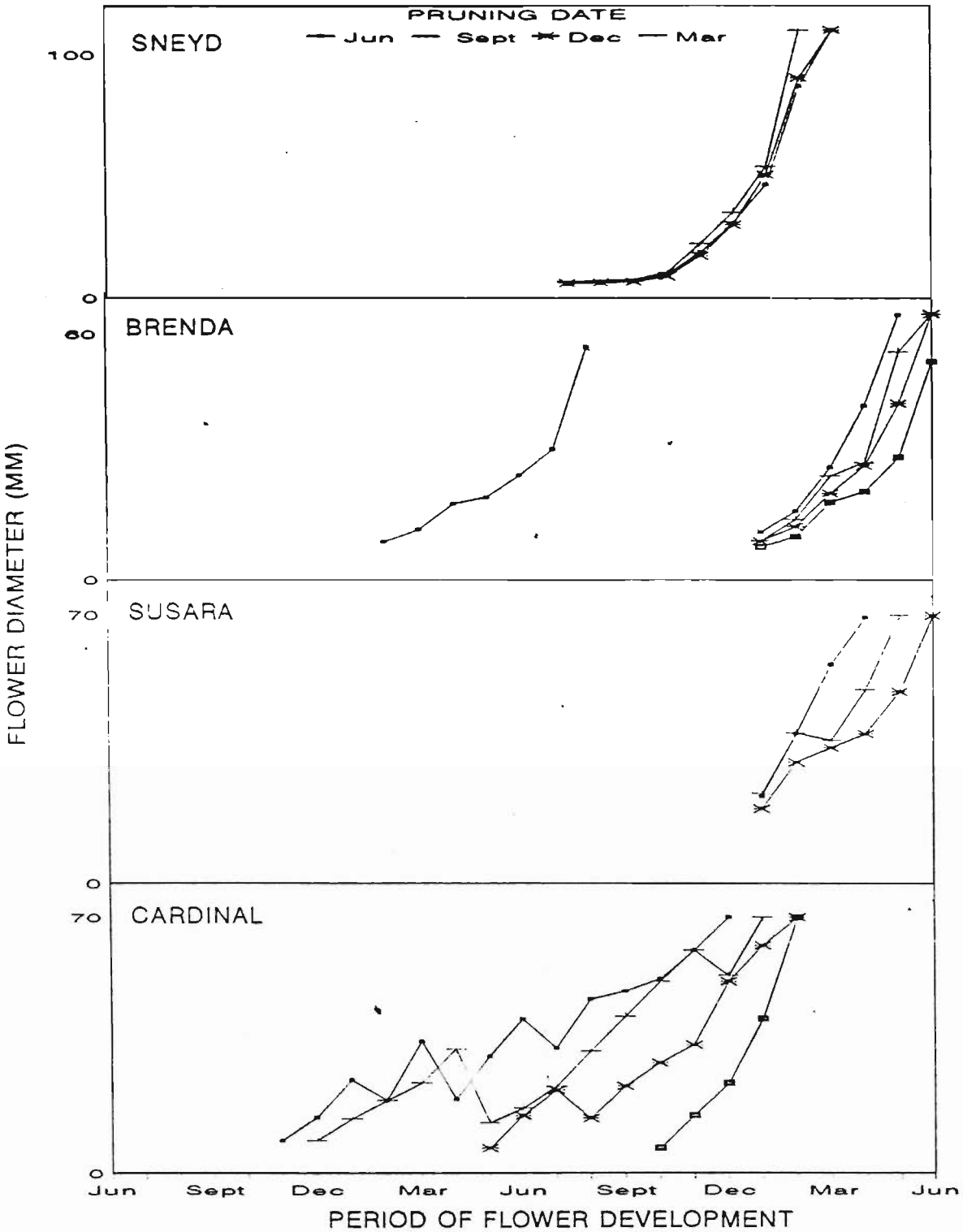

Figure 2. Inflorescence development (diameter measured) of *Protea* cultivars following pruning on four different dates as indicated. Treatments consisted of 25 single shoot replicates and data represent the length only of flowering shoots.

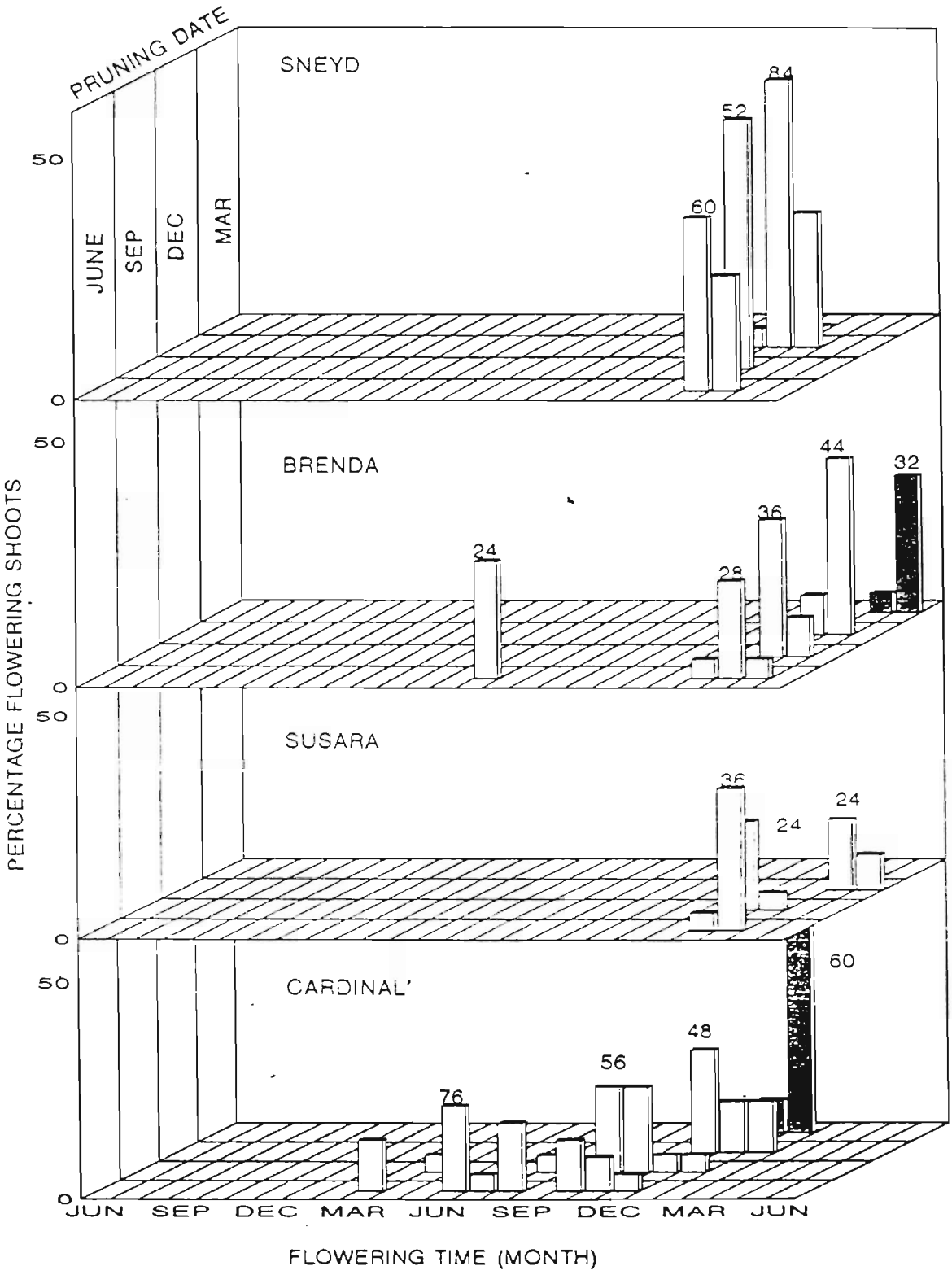

Figure 3. Flowering time and percentage flowering within the experimental period (figure above tallest bar) of *Protea* cultivars following pruning on four different dates. Treatments consisted of 25 single shoot replicates.

PRUNING OF PROTEA cv. CARNIVAL TO OPTIMISE ECONOMIC BIOMASS PRODUCTION

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Abstract

Plants of *Protea* cv. Carnival were pruned on six different dates in 1991. The 1992 and 1993 yields were analysed in terms of vegetative, reproductive and economic biomasses. The pruning date influenced biomass allocation, and determined whether flowers were produced annually or biennially. Pruning in March, April or May resulted in an annual flowering cycle, with less than 40% of the fresh mass produced being reproductive. Pruning in July, August or September resulted in up to 70% reproductive biomass being produced in a biennial cycle. The biennial cycle resulted in an earlier harvest.

1. Introduction

Proteas grown for export to the European cut flower market must be cultivated with emphasis on quality and time of production. Cut flower proteas are in greater demand and command better prices from September to May. During the remaining months (European summer), there is great competition from cut flowers grown in Europe, thus reducing demand for imported flowers. Further, profit is decreased by higher import surcharges imposed on cut flowers imported during the European summer. Competition between commercial protea growers results in high prices being paid only for best quality blooms, even during times of high demand.

Pruning of proteas releases lateral buds from apical dominance, resulting in growth of vegetative shoots which elongate by successive growth flushes. Elongation stops when flower initiation occurs terminally. *Protea* cv. Carnival (a natural hybrid, possibly between *P. neriifolia* and *P. compacta*) produces an autumn flush, starting in April/May, a spring flush, starting in August/September, and two summer flushes, the first beginning in December, and the second in February to April (Greenfield *et al.*, 1993). According to Dupee and Goodwin (1990) flower initiation in *P. neriifolia* occurs after growth of the spring flush is complete. However, Greenfield *et al.* (1993) found that vegetative shoots of *Protea* cv. Carnival initiated flowers terminally on the spring or first summer growth flushes. Fifty percent of the flowers were initiated on spring flushes, at which stage stems generally consisted of only two or three growth flushes. Current pruning practices aim at producing both shoots and flowers in the same year. This results in low yields of short-stemmed flowers. In this paper we report on yield and flower quality as affected by pruning practices which result in shoot growth in one year and flowers the following year.

2. Material and methods

Six-year-old plants of *Protea* cv. Carnival grown commercially under natural climatic

conditions near Stellenbosch, Cape Province, South Africa (33° 54'S) were used. The area receives an annual winter rainfall of 600 — 700 mm. Plants were spaced one metre apart in the row and four metres between rows and were not irrigated or fertilized. In 1991 plants were pruned on six different dates, namely, 12 March, 9 April, 21 May, 2 July, 13 August, and 17 September. Plants pruned on the first three dates (referred to as 'early pruning') flowered the following season (1992), and were pruned again on 24 April 1992, when harvesting was complete. Plants pruned on the last three dates (referred to as 'late pruning') failed to flower in 1992 and were not pruned in 1992.

Pruning entailed heading both flowering and non-flowering shoots, leaving a 15 cm portion of the stem to serve as a bearer for the following year's growth. Spindly shoots were removed by thinning cuts. In 1991 the number of bearers per plant was reduced using thinning cuts to leave 2.5 bearers per cm trunk circumference, as measured 10 cm above ground level. In following years the number of bearers was not specified and counted, but spindly growth was thinned out, as is done in commercial practice.

Flowers produced in 1992 and 1993 were harvested when commercially mature. In 1992 flowers were harvested for maximum stem length, as done commercially, leaving either a short bearer or with a thinning cut. In 1993 flowers were harvested leaving a 15 cm bearer, regardless of the length of the stem. Once harvesting was complete plants were pruned as described above. Flowering and non-flowering shoots were weighed to determine reproductive and vegetative biomass respectively. Shoots that developed below a flowerhead were cut off and included in the vegetative biomass. Flowering stems were classified according to stem length and quality. Stems shorter than 50 cm were classified as non-exportable. Percentage reproductive biomass was calculated as the percentage of the total biomass that consisted of flowering shoots, without taking flower and stem quality into account.

Single plants were used per treatment, replicated 10x in a randomised complete block design. Data was analysed using the GLM proceedure of SAS.

3. Results

The time of pruning dictated whether the plant produced both shoots and flowers in the same year or produced shoots only in the first year followed by flowers the next year. Plants which were pruned early in 1991 and again in 1992 produced both shoots and flowers in the same year. Flowers were produced annually in 1992 and 1993. Plants which were pruned late in 1991 and not pruned in 1992 produced shoots only during the 1991/1992 growing season and flowers during the following season. This shows a trend towards biennial flower production.

3.1. Fresh biomass production

Total fresh biomass, reproductive biomass, and percentage reproductive biomass produced in 1992 were all significantly decreased by delaying pruning from March to May 1991 (table 1). Vegetative biomass production was not affected by date of pruning.

Plants flowering biennially produced significantly more total, reproductive, and percentage reproductive biomass in 1993 than plants flowering annually (table 2). The only carry-over effect of the 1991 pruning on the 1993 harvest (with regard to early-pruned plants) was in the percentage reproductive biomass, where plants pruned

in May 1991, had a higher percentage than plants which were pruned earlier.

Comparing the combined biomass removed in 1992 and 1993 from plants flowering annually with the biomass removed in 1993 from plants flowering biennially showed significant differences in all but the total biomass (table 3). Plants flowering annually produced more vegetative biomass and less reproductive biomass, resulting in a lower percentage reproductive biomass.

3.2. Yield and flower quality

The total number of flowers produced in 1992 by early-pruned bushes was reduced linearly by delaying pruning from March to May (table 4). Fifty percent of the flowers had stems shorter than 50 cm.

Plants which were not pruned in 1992 (biennial flowering) produced significantly more flowers in 1993 than plants which were pruned in 1992 (annual flowering) (table 5). Of the plants flowering biennially those pruned in September 1991 produced the most flowers. The majority of the flowers produced in 1993 by plants flowering annually had stems shorter than 50 cm. In contrast, more than 70% of flowers produced biennially in 1993 had stems longer than 50 cm.

Plants flowering biennially produced more flowers in 1993 than the combined 1992/1993 harvest from plants which flowered in both years (table 6). Thus over a two year period plants pruned in July, August or September 1991, produced more flowers than plants pruned in March, April or May 1991 and again in April 1992. Delaying pruning until late 1991 shifted the harvest from flowers produced annually with short stems (less than 50 cm) to flowers produced biennially with long stems (longer than 50 cm).

3.3. Time of harvest

The time of harvest was affected by the date of pruning in 1991 (table 7). Plants flowering annually produced the most flowers in April, with less than 40% being picked in March and only a small percentage in February. The biennial harvest in 1993 was early, with approximately 60% of the harvest being picked in February. Harvesting began in January and continued until 12 March when the plants were finally pruned.

4. Discussion

With present pruning practices *Protea* cv. Carnival has an annual flowering cycle. Stems elongate following budbreak shortly after pruning until late spring or early summer when flower initiation takes place. Two to three flushes are produced during this 8 to 9 month growing period. By changing the time of pruning the cycle can be adjusted to a biennial one. The advantage of biennial flowering over annual flowering in terms of time, size and quality of harvest is due solely to the fact that flower initiation does not take place in the first year of the biennial cycle.

It is not known what factor(s) control flower initiation in *Protea* cv. Carnival. Flower initiation is induced by short days in *Leucospermum* cv. Red Sunset (Malan and Jacobs, 1990) and *Serruria florida* (Malan and Brits, 1990). De Swardt (1989) found that no shoot growth or reproductive development occurred in *Protea* cv. Ivy under

short day conditions, although shoot growth continued in winter when long days were simulated with artificial lighting (unpublished data). It is unlikely that flower initiation in *Protea* cv. Carnival is affected by day-length alone since flowers can be initiated on the first summer flush, produced in mid-summer (December), but not the second summer flush, produced in late summer.

De Swardt (1989) found that the length and thickness of stems of *Protea* cvs. Ivy and Carnival significantly contributed to the ability of the stem to produce a flower, although this was not the only factor involved. Longer, thicker stems were more likely to produce flowers than short, thin stems. Greenfield *et al.* (1993) suggested that at least two flushes of shoot growth are needed before flower initiation will occur in *Protea* cv. Carnival. In the first year of the biennial cycle shoots had elongated by only one or two growth flushes at the time at which flower initiation normally takes place. These shoots were probably below a critical stem length or diameter necessary for flower initiation. Shoot elongation continued into the second year of the biennial cycle when flowers were initiated, by which time stems were long enough for export. Stem diameter also plays a role in flower quality — an increase in diameter leading to an increase in flowerhead dry mass (Napier *et al.*, 1986), flowerhead diameter and number of styles (Jacobs and Minnaar, 1980; Jacobs, 1983).

Greenfield *et al.* (1993) found that in an annual cycle low yields of *Protea* cv. Carnival were not due to a shortage of shoots. Many of the shoots probably did not have the stem length or diameter characteristics supposed to be necessary for flower initiation (de Swardt, 1989). In the biennial cycle these shoots are allowed a further growing season in which to lengthen and thicken before producing flowers. This explains the difference in percentage reproductive biomass between plants flowering annually and biennially while the total biomass produced did not vary.

Shoots produced by plants flowering biennially were long and thick in spring of the second year. Flowers were initiated on the spring flush and matured early.

Biennial flower production improves both yield and flower quality. Flowers are mature for harvesting early in the year (southern hemisphere) when demand is high. The improved harvest in terms of time, numbers, and quality more than compensates for cropping only every second year.

Acknowledgements

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Table 1. Effect of pruning date on fresh biomass production of *Protea* cv. Carnival – 1992 harvest.

Pruning date 1991	Total Mass (g)	Reprod Mass (g)	Veget Mass (g)	% Reprod Mass
Annual harvest				
12 March	6936	2471	4465	37
9 April	5194	1677	3518	30
21 May	4023	752	3271	17
Mean	5384	1633	3751	28
Biennial harvest				
2 July	–	0	–	0
13 August	–	0	–	0
17 September	–	0	–	0
ANOVA				
Source	Significance			
Date of pruning LIN	0.0105	0.0012	0.0964	0.0017
Date of pruning Quad	0.8189	0.0349	0.8040	0.1728

Table 2. Effect of pruning date on fresh biomass production of *Protea* cv. Carnival — 1993 harvest.

Pruning date		Total Mass (g)	Reprod Mass (g)	Veget Mass (g)	% Reprod Mass
1991	1992				
Annual harvest					
12 March	24 April	7915	2144	5772	25
9 April	24 April	6276	1251	5025	19
21 May	24 April	7900	3162	4738	40
Mean		7364	2186	5178	28
Biennial harvest					
2 July	none	12531	8195	4336	66
13 August	none	12557	8772	3785	71
17 September	none	14432	10110	4322	70
Mean		13173	9025	4148	69
ANOVA					
Source		Significance			
Annual *vs* Biennial		0.0001	0.0001	0.0339	0.0001
Annual LIN		0.9088	0.2836	0.2304	0.0001
Annual QUAD		0.2829	0.1781	0.6390	0.0005
Biennial LIN		0.9485	0.4055	0.2111	0.0007
Biennial QUAD		0.2780	0.1301	0.7944	0.0001

Table 3. Effect of pruning date on fresh biomass production of *Protea* cv. Carnival — combined harvest.

	Pruning date		Total Mass (g)	Reprod Mass (g)	Veget Mass (g)	% Reprod Mass
	1991	1992				
Annual mean	Early	24 April	12742	3809	8934	29
Biennial mean	Late	None	13173	9032	4141	69
ANOVA						
Source			Significance			
Annual *vs* Biennial			0.7293	0.0001	0.0001	0.0001

Table 4. Effect of pruning date on flower quality of *Protea* cv. Carnival — 1992 harvest.

Pruning date 1991	Number stems	Percentage of total		
		> 50 cm	30 — 50 cm	< 30 cm
Annual harvest				
12 March	18	44.2	56.6	0.0
9 April	14	35.3	64.2	0.5
21 May	7	63.3	22.8	4.0
Mean	13	47.6	47.9	1.5
Biennial harvest				
2 July	0			
13 August	0			
17 September	0			

ANOVA

Source	Significance
Date of pruning LIN	0.0116
Date of pruning QUAD	0.9155

Table 5. Effect of pruning date on flower quality of *Protea* cv. Carnival — 1993 harvest.

Pruning date		Number stems	Percentage of total		
1991	1992		> 50 cm	30 — 50 cm	< 30 cm
Annual harvest					
12 March	24 April	15	4.7	78.3	16.6
9 April	24 April	11	6.0	82.4	11.1
21 May	24 April	26	3.8	86.6	8.3
Mean		17.3	4.8	82.4	12.0
Biennial harvest					
2 July	none	45	74.2	10.7	0.0
13 August	none	56	79.6	12.6	0.6
17 Sept	none	63	85.8	8.2	0.1

ANOVA

Source	Significance
Annual *vs* Biennial	0.0001
Annual LIN	0.0382
Annual QUAD	0.0999
Biennial LIN	0.0756
Biennial QUAD	0.0483

Table 6. Effect of pruning date on flower quality of Protea cv. Carnival — combined harvest.

Pruning date		Number stems	Percentage of total		
1991	1992		> 50 cm	30 — 50 cm	< 30 cm
Annual harvest					
12 March	24 April	32	27.3	65.9	6.6
9 April	24 April	25	21.5	73.7	4.5
21 May	24 April	34	15.4	76.4	7.3
Mean		30.3	21.4	72.0	6.1
Biennial harvest					
2 July	none	45	74.2	10.7	0.0
13 August	none	56	79.6	12.6	0.6
17 Sept	none	63	85.8	8.4	0.1
Mean		54.7	79.8	10.6	0.2

ANOVA

Source		Significance
Annual vs Biennial		0.0001
Annual	LIN	0.7465
Annual	QUAD	0.1862
Biennial	LIN	0.9215
Biennial	QUAD	0.1721

Table 7. Effect of pruning date on time of harvest of *Protea* cv. Carnival — combined harvest.

Pruning date		January %	February %	March %	April %
1991	1992				
Annual harvest					
12 March	24 April	0	3.84	38.44	57.75
9 April	24 April	0	3.65	36.86	59.48
21 May	24 April	0	3.41	36.12	60.46
Mean		0	3.63	37.14	59.23
Biennial harvest					
2 July	none	3.55	60.11	36.35	0
13 August	none	0.61	58.85	40.54	0
17 Sept	none	1.94	63.05	35.00	0
Mean		2.03	60.67	37.30	0

EFFECT OF TWO IRRIGATION FREQUENCIES ON WATER STATUS, LEAF DIFFUSIVE CONDUCTANCE AND NET PHOTOSYNTHESIS IN *PROTEA EXIMIA* GROWN ON GRAVEL SUBSTRATE

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Abstract

Protea eximia seedlings were grown on porphyry substrate in soilless greenhouse culture to study the effect of irrigating 2 and 6 times daily in summer. In both treatments, stomatal regulation appeared very efficient in maintaining constant water content in leaves picked at noon. The highest leaf diffusive conductance, net photosynthesis and production of dry and fresh matter correlated with the highest watering frequency, and conversely. Nonetheless, even the highest watering frequency could not prevent a gradual decrease in leaf diffusive conductance throughout the day. This can be attributed to porphyry's low water retention capacity, or the insufficient frequency of irrigation. Several hypotheses are presented to explain low predawn water potential values (around -0.4 MPa).

1. Introduction

Protea belongs to a botanical family from the southern hemisphere. Their unique appearance and the exceptional diversity of their forms and colours compared to traditional cut flowers have attracted considerable interest (Urban and Allemand, 1992). In addition, the cut flowers are remarkably long-lasting and can be kept for up to 3 weeks. The development of Proteaceae for cut flower production in Europe is limited, however, because of the plants' sensivity to limestone in the soil and to frost (Vogts, 1982). Consequently, it is necessary to consider growing these plants under soilless greenhouse conditions.

Soilless culture requires fertilizing irrigation to be adjusted. The purpose of this work was to determine the effect of frequency of irrigation on the water status and net photosynthesis of *Protea eximia* (Salisb. ex Knight) Fourcade grown in prophyry in soilless conditions.

2. Materials and methods

2.1. Experimental layout

The trial was conducted on 3-year-old *P. eximia* seedlings in an inflated double-walled plastic greenhouse (Celloflex EVA by Prosyn-Polyane). The plants had been topped after development of the 5th growth flush. Sown in January 1989, the seedlings were planted in July 1989, 2 plants.m^{-2} in polypropylene tubs (300 cm x 90 cm x 18 cm) containing porphyry from the Estérel mountains (5 to 7 mm diameter). Although this substrate provides excellent aeration, its water retention is poor. Climatic regulation was provided by opening the windows when the temperature of the air in

the greenhouse rose above 20°C. The experimental layout was made up of 4 blocks x 2 treatment groups (split plots), each component plot comprising 6 seedlings. The plots were standardized according to the initial number of leaves/seedling. Measurements were made from July 9 to August 21, 1992.

2.2. Fertilizing irrigation

Fertilisation was applied as described by Montarone (1992). Electrical conductivity (EC) of the nutrient solution was maintained at 900 ± 100 $mS.cm^{-1}$, and pH at 7 ± 1. Irrigation was controlled by a timer. The characteristics of both treatments are shown in table 1. EC and pH were checked daily around noon on drainage water, using portable apparatus (HI 8733, Hanna for EC, and Quick 93314, Bioblock Scientific for pH). The water potential of the drainage solution (Y_{drain}) was calculated using Ayers and Westcot's (1975) formula.

2.3. Water status measurements

Water status was assessed by measuring water content of 12 mature leaves picked every working day around noon, WC_{noon} (1 leaf per treatment and per block). Relative water content could not be measured since the samples placed in saturation chambers were observed to continue increasing in weight, even after 48 hours. WC_{noon} may be considered to measure the plants' maximum water stress. Predawn water potential ($Y_{predawn}$) was measured each 5 days by psychrometry (C52 chambers connected to a Wescor HR33T microvoltmeter) on one mature-leaf foliar disc per treatment. $Y_{predawn}$ may be considered to measure the plants' minimum water stress.

2.4. Gas exchange measurements in leaves

Net photosynthesis and leaf diffusive conductance were monitored using a portable, open-system, infrared gas analyser LCA-2 (ADC) and a leaf chamber (Parkinson, 1983). About 20 s were required to calibrate CO_2 depletion in the differential mode. All measurements were performed with young mature leaves oriented perpendicular to the sun on 9 clear days selected for the sake of homogeneity (Urban and Langelez, 1992). Calculations were made using Von Caemmerer and Farquhar's (1981) method. Measurements were made at 6 am, 8 am, 11 am, 2 pm and 4 pm, on two leaves per treatment and per block.

2.5. Production

At the onset of the trial, all buds were tagged. The production of dry and fresh matter was subsequently measured for each treatment.

3. Results

3.1. Effect of irrigation frequency on water availability in the substrate and on the water status of plants

In both treatments, Y_{drain} values were very high (very close to zero) (table 2),

while $Y_{predawn}$ values seemed rather low (negative), around -0.4 MPa. There was no significant difference in Y_{drain} or $Y_{predawn}$ values between both treatments. WC_{noon} was not significantly affected by any of the two irrigation schedules (table 2).

3.2. Effect of irrigation frequency on leaf diffusive conductance and net photosynthesis

Leaf diffusive conductance started to decrease at 6 am in treatment 1, and at 8 am in treatment 2 (figure 1). It did not differ significantly between both treatments at 6 am, but was significantly lower in treatment 1 than in treatment 2 from 8 am to 4 pm. Net photosynthesis was not proportional to leaf diffusive conductance (figures 1 and 2). In treatment 2, it followed solar radiation, increasing from 6 am to peak at 11 am (figures 2 and 3), but had fallen by 11 am in treatment 1. Net photosynthesis was significantly higher between 8 am and 4 pm in treatment 2 than in treatment 1.

3.3. Effect of irrigation frequency on production

Significantly larger amounts of dry and fresh matter were produced in treatment 2 than in treatment 1 (table 3).

4. Discussion

The stomata play a regulatory role in plants' water status by adjusting transpiratory flow to the availability of water in the root environment (Schulze, 1986). Stomatal conductance indeed appeared highest in the highest irrigation frequency treatment, and conversely (figure 1). Stomatal regulation seems to be remarkably efficient in *P. eximia* in maintaining the same leaf water content, irrespective of the water availability in the root environment (table 2). It is likely that stomatal regulation is the key survival process for these plants in arid conditions.

Even in the highest irrigation frequency treatment, there was a decrease in stomatal opening by 8 am (figure 1). This shows that irrigation was not optimal in our trial. We believe the problem lies with porphyry's low water retention capacity and/or the insufficient frequency of watering rather than with the total amount of supplied water.

Between 8 am and 4 pm, stomatal conductance and net photosynthesis were highest in the highest irrigation frequency treatment and lowest in the lowest irrigation frequency treatment (figures 1 and 2). It thus appears that frequent irrigation promotes net photosynthesis by improving stomatal opening. Nonetheless, it is also obvious that the photosynthesis was not proportional to stomatal conductance: the former increased between 8 am and 11 am, while the latter decreased. Farquhar and Sharkey (1982) had already shown that net photosynthesis and stomatal conductance were not proportional.

The improved net photosynthesis in the highest irrigation frequency treatment was clearly expressed by an increase in the dry and fresh matter produced in comparison with treatment 1 (table 3).

Surprisingly, Y_{drain} values were higher (less negative) than $Y_{predawn}$ values (table 3). The latter seem very low (very negative) compared to the $Y_{predawn}$ measured, for instance, on roses grown in rockwool slabs (data not shown). This difference shows

the existence of a Y gradient between the substrate near the roots and elsewhere. This gradient could be the result of secretions by the proteoid roots of the Proteaceae (Gardner *et al.*, 1982) which tend to acidify the solution, decompose the porphyry and increase ion concentration near the roots (Montarone, 1992). It can also be hypothesized that porphyry's low water retention capacity is the cause of the rapid decrease in the amount of water available near the roots at night, if the plants are not watered. The matricial potential which retains water in the substrate then tends to decrease, leading to low $Y_{predawn}$ values.

Finally, we are not absolutely certain that the mature leaves used to measure $Y_{predawn}$ did not continue growing during the night. It should be noted that growth is expressed by a decrease in water potential (Boyer, 1970; 1985).

5. Conclusion

This work has helped to reveal the efficiency of stomatal regulation in *P. eximia*, along with the favourable effect of increased frequency of irrigation on stomatal opening, net photosynthesis and production. Further improvement in *P. eximia* production in soilless conditions should result from the substitution of porphyry by a substrate which can resist acidification of the medium and/or has a better water retention capacity (like rockwool).

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Table 1. Characteristics of the two irrigation schedules applied to *Protea eximia* on porphyry substrate.

Treatment	Daily number of water supplies	Average daily volume of water supply ($\ell.m^{-2}$)	Average drainage rate (%)
1	2	5.6	11.2
2	6	12	47.7

Table 2. Effect of irrigation schedules on water stress experienced by *Protea eximia* on porphyry substrate.

Treatment	$Y_{predawn}$ (MPa)	Y_{drain} (MPa)	WC_{noon} (%)
1	-0.45	-0.015	70.4
2	-0.43	-0.014	70.0

Means not significantly affected by treatments (t test at $P=0.05$).

Table 3. Effect of irrigation schedules on fresh and dry matter production of *Protea eximia* on porphyry substrate.

Treatment	Dry matter production ($kg.m^{-2}$)	Fresh matter production ($kg.m^{-2}$)
1	0.16a	0.62a
2	0.18b	0.69b

Means within columns not sharing the same letter are significantly different by t test with $P < 0.05$.

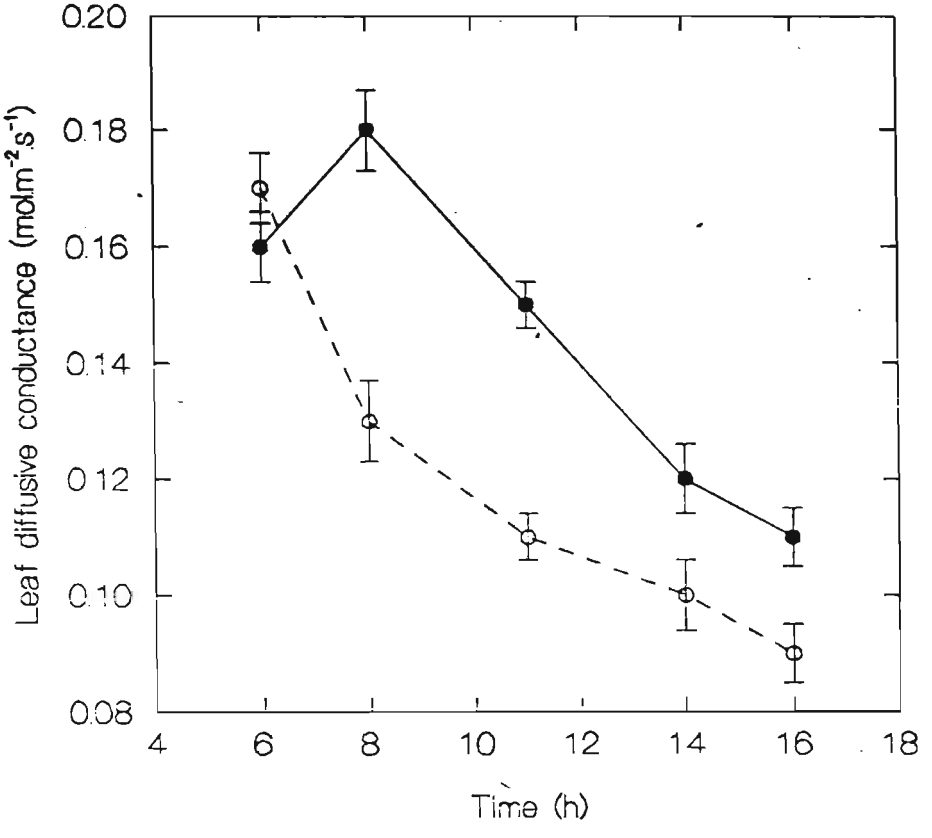

Figure 1. Daily variation of leaf diffusive conductance. Each point represents the average over 72 leaves (9 days x 8 leaves per treatment for each selected hour). The dashed line corresponds to the low irrigation frequency, and the full line to the high irrigation frequency. Vertical bars indicate standard deviations.

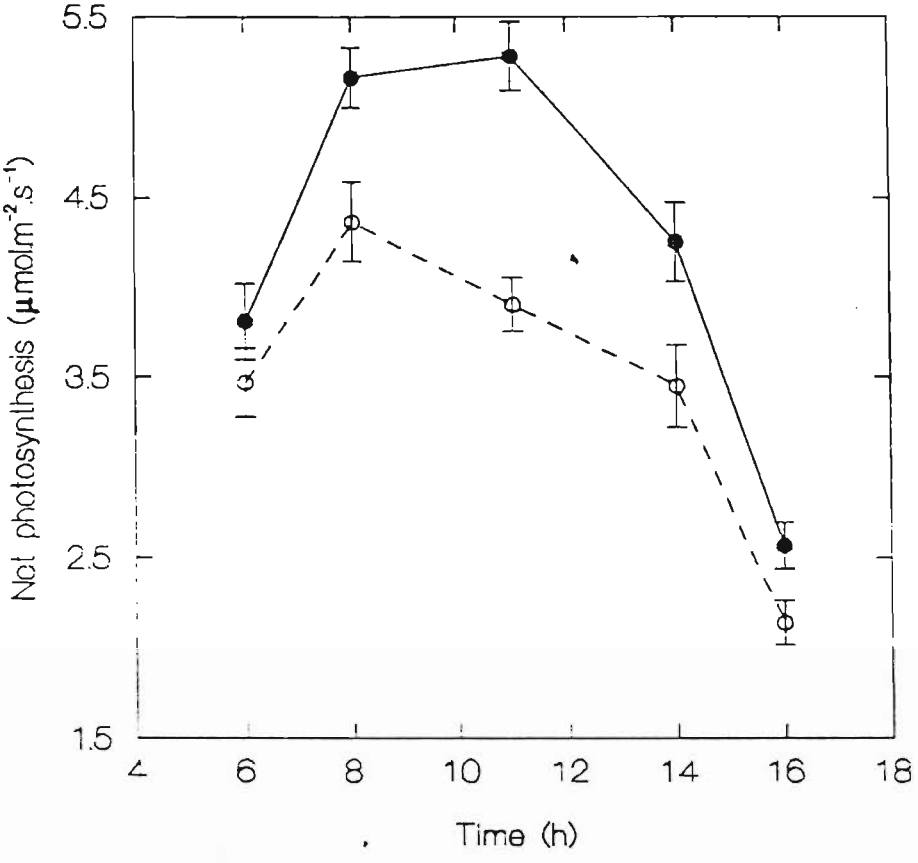

Figure 2. Daily variation of net photosynthesis. Each point represents the average over 72 leaves (9 days x 8 leaves per treatment for each selected hour). The dashed line corresponds to the low irrigation frequency, and the full line to the high irrigation frequency. Vertical bars indicate standard deviations.

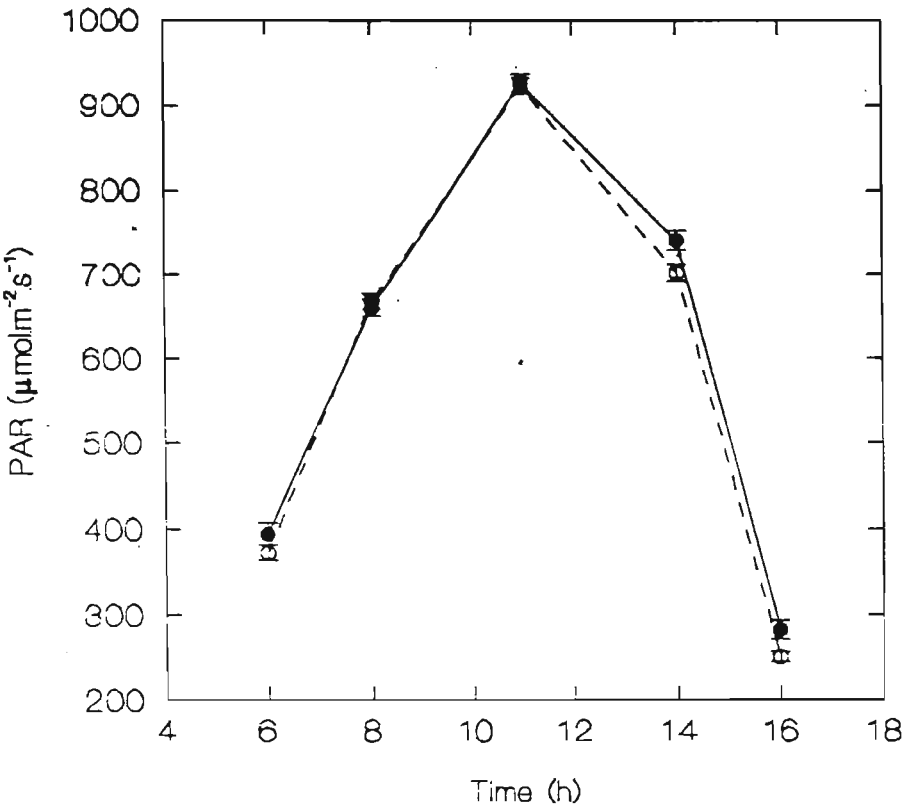

Figure 3. Daily variation of photosynthetically active radiation (PAR). Each point represents the average over 72 measurements (9 days x 8 measurements per treatment for each selected hour). The dashed line corresponds to the low irrigation frequency, and the full line to the high irrigation frequency. Vertical bars indicate standard deviations.

EFFECT OF DIFFERENT FACTORS ON *IN VITRO* MULTIPLICATION OF *LEUCADENDRON* 'SAFARI SUNSET' (PROTEACEAE)

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Abstract

Some factors affecting the multiplication phase of micropropagation of *Leucadendron* 'Safari Sunset' through nodal segments were studied. The presence of 6-benzyladenine (BA) in a multiplication medium resulted in a higher proliferation rate than kinetin or 2-isopentenyl-adenine (2-iP). The use of a mixture containing BA and kinetin produced a synergistic effect on bud formation. The presence of naphthaleneacetic acid (NAA), indole-butyric acid (IBA) or gibberellic acid in the proliferation medium containing BA, did not significantly affect the formation of new buds per explant and its growth. The MS medium at half strength produced better bud formation than $^3/_4$ MS and complete MS media. The presence of activated charcoal in the medium clearly inhibited bud proliferation while the use of phytagel as agar substitute gelling agent produced a significant increase of bud multiplication and growth.

1. Introduction

Leucadendron 'Safari Sunset' (Proteaceae) is the most promising protea cultivar for Tenerife, due to its fast growth and early entrance in production. The demand for plants of this protea is high, but the local offer is low. Although a certain number of plants has been imported from other countries (Israel, USA), the cost of each plant is high (about 3.00 $ USA). In order to overcome this problem, the study of micropropagation of *Leucadendron* 'Safari Sunset' was begun. It is known that micropropagation could be a good alternative technique to commercial production of selected cultivars and the micropropagation of some protea species has been reported (Ben-Jaacov and Dax, 1981; Bunn and Dixon, 1992a, 1992b; Kunisaki, 1989; Malan, 1992; Seelye *et al.*, 1986; Offord and Campbell, 1992; Offord *et al.*, 1992). The present study reports on a series of treatments made for *in vitro* multiplication from axillary buds of *Leucadendron* 'Safari Sunset'.

2. Material and methods

Field grown shoots (20 — 30 cm in length) were taken from 3-year-old plants of *Leucadendron* 'Safari Sunset'. Collections were made during July 1991 from the plantation of Florican (Los Rodeos, La Laguna, Tenerife). Selected shoots (about 10 cm in length) were cut and pre-sterilized by immersion in a mix of 0.5 g.ℓ^{-1} Benlate (benomyl 50% a.i.), 1 g.ℓ^{-1} Orthocide (captab 50% a.i) and 0.1% Tween-20 for 1 h, washed and then surface-sterilized in a solution of 4% Ca-hypochlorite plus 0.1% Tween-20 for 10 min with gentle agitation. Partially lignified nodal segments (1.5 — 2.0 cm in length) were excised from the shoots, leaves were removed and shoots were re-sterilized under vacuum in 1% Ca-hypochlorite also with 0.1% Tween-20 for 30 min.

Afterwards, they were rinsed four times in sterile distilled water and kept in a shaking, sterile solution of citric acid (1500 mg.ℓ^{-1}) and ascorbic acid (100 mg.ℓ^{-1}) for at least 1 h to avoid browning oxidation. This mixture was also included in the medium during the establishment phase.

MS medium (Murashige & Skoog, 1962) with 2% sucrose were used. See legends of figures for more detail about culture media.

For the establishment of cultures, single node explants were cultured on 20 mℓ of MS medium without BA in 24 x 160 mm tubes. Within 20 days explants developed axillary buds. These were subcultured to a MS medium containing 0.89 μM BA for axillary bud elongation. After 30 — 40 days, buds were excised and 4 buds were planted per glass culture vessel (72 mm height) with Magenta B-caps (Sigma Co.) containing 20 mℓ of medium. For shoot multiplication, segments of about 1 — 2 cm were transferred monthly to a fresh medium.

Cultures were kept at 26 °C ± 1 and a photoperiod of 16 h (110 μE.m^{-2}.s^{-1}) provided by white fluorescent tubes (Philips TLD, 58W/84) and 50 — 60% humidity.

3. Results

3.1. Effects of ionic strength, activated charcoal and gelling agent

The MS medium at half-strength produced a higher mean number of buds per explant than $^3/_4$ MS or complete MS medium. However the bud growth was not significant affected (figure 1).

The presence of activated charcoal in the multiplication medium clearly inhibited bud proliferation. Bud growth was also decreased with this treatment (figure 1).

The use of phytagel as agar substitute gelling agent produced a significant increase of bud multiplication and growth. Bud multiplication decreased with further subcultures through the multiplication phase except when phytagel was used (figure 1).

3.2. Effect of growth regulators

In previous work (unpublished results), the use of 0.89 μM BA produced 6 — 8 axillary buds per explant during several subcultures and we have used it as a control. The presence of kinetin or 2iP as a BA substitute cytokinin in the multiplication medium resulted in a substantially lower number of buds per explant than using 0.89 μM BA (figure 2a). A mean of only 1.5 to 3 buds per explant were observed with these cytokinins. The use of a mixture containing BA and kinetin produced a synergistic effect on shoot formation but the mean number of buds per explant decreased significantly at the third subculture (figure 2a).

Figure 2b shows the increase in the mean length of shoots when different cytokinins were included in the culture medium. No pronounced differences in bud growth were observed among the different cytokinin treatments used in this report. After the second subculture to the same proliferation medium, the growth rate of axillary buds markedly decreased.

The presence of NAA or IBA in the proliferation medium containing BA, did not significantly affect the formation of new buds per explant and its growth. A slightly inhibitory effect on bud multiplication, especially at the third subculture was detected

with NAA (figure 3).

The use of GA_3 (0.29 and 2.89 μM) did not significantly affect the multiplication and growth of buds. A combination of BA, NAA and GA_3 in the proliferation medium produced similar results to the medium with only BA (figure 3).

4. Discussion

BA is the most suitable cytokinin for axillary shoot multiplication of *Leucadendron* 'Safari Sunset' from partially lignified nodal segments. A mixture of 0.89 μM BA and 0.89 μM kinetin produced a synergistic effect on bud proliferation and growth. However, further investigation is needed to establish the most effective concentrations of this cytokinin or others for the multiplication phase. On the other hand, shoot elongation was best using kinetin, but only at the first subculture. Under our experimental conditions shoots grew slowly from bud clusters obtained through the axillary zones of the multiplication phase. Incubating explants in a liquid-shaking system for a brief period has been reported to produce a modification of the physiological state of certain plants, increasing the proliferation rate during the multiplication phase (Snir and Erez, 1980). We tried this method. Unfortunately, a very high percentage of explants became brown within a few days (data not shown).

Several reports have showed that agar quality can strongly influences *in vitro* shoot proliferation (Singha, 1984) and the growth response of shoot explants *in vitro* (Pierik, 1991). We found that an important factor for the multiplication phase of *Leucadendron* 'Safari Sunset' was the use of phytagel.

In conclusion, of the treatments examined in this study, the use of BA as the most suitable cytokinin, the negative response with the addition of NAA, IBA and/or GA_3, the inhibitory effect of activated charcoal, the beneficial effect of a half-strengh MS medium and the use of phytagel appear to be of importance for the multiplication of axillary shoots of *Leucadendron* 'Safari Sunset' *in vitro*. However, conditions during the multiplication phase must be refined, to achieve a greater multiplication rate. In addition, the use of relatively high concentrations of cytokinins must be evaluated for studying somaclonal variation in regenerated plants.

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Figure 1a

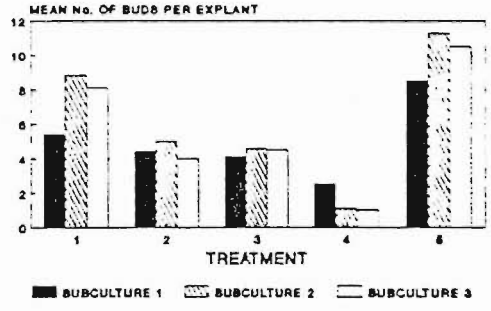

Figure 1b

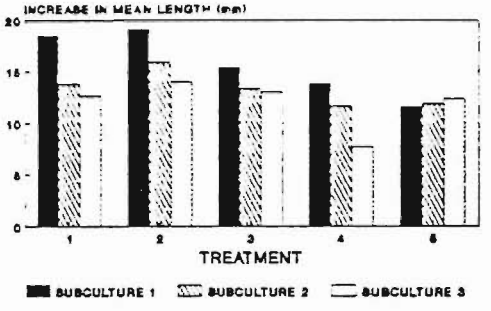

Figure 1. Effect of several factors in the multiplication media on the mean number of axillary buds per explant (a) and on the increase in the mean length of axillary shoots (b) during 3 subcultures to the same medium. The basic culture medium was the MS medium with 0.89 μM BA, 2% sucrose and pH 5.8. Five medium treatments were applied: 1 = ½ macrosalts of MS medium, 2 = $^3/_4$ macrosalts of MS medium, 3 = complete MS medium, 4 = 0.3% (w/v) activated charcoal and 5 = 2 g.ℓ^{-1} phytagel as agar substitute gelling agent.

Media of treatments 1 to 4 were solidified with 7 g.ℓ^{-1} agar (Sigma No. A-7002).

Figure 2a

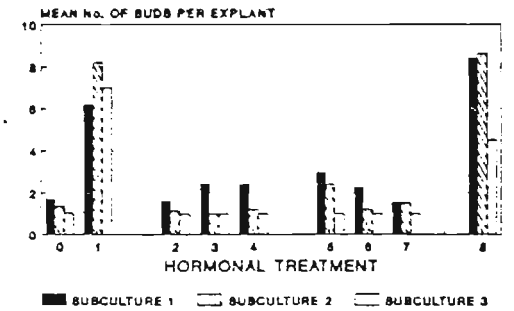

Figure 2b

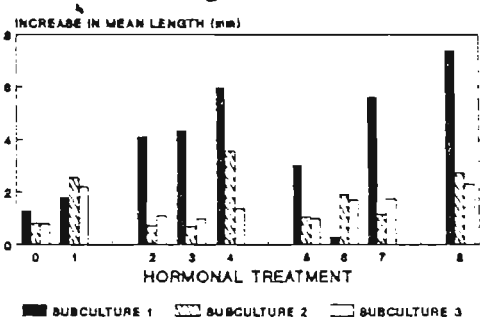

Figure 2. Effect of multiplication media containing different cytokinins on the mean number of axillary buds per explant (a) and on the increase in the mean length of axillary shoots (b) during 3 subcultures to the same medium. The basal medium was the MS medium with macro-elements at half strength supplemented with 0.7% agar, 2% sucrose, pH = 5.8 and growth regulators as indicated below.

Growth regulator treatments: 0 = without hormones, 1 = 0.89 μM BA (used as control), 2 = 0.89 μM kinetin, 3 = 2.22 μM kinetin, 4 = 4.40 μM kinetin, 5 = 0.89 μM 2-iP, 6 = 2.22 μM 2-iP, 7= 4.40 μM 2-iP and 8 = 0.89 μM BA + 0.89 μM kinetin.

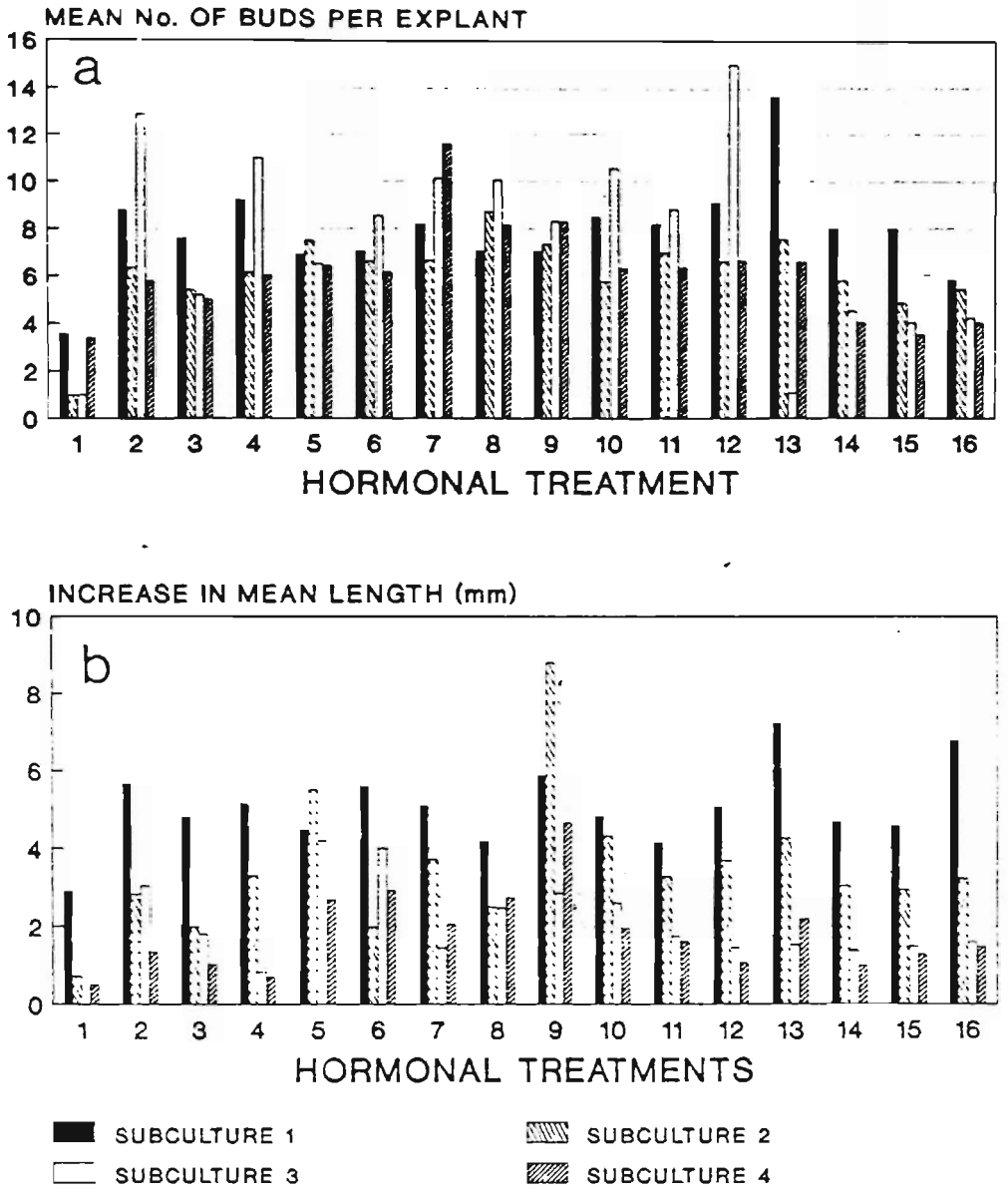

Figure 3. Effect of multiplication media containing different growth regulators on the mean number of axillary buds per explant (a) and on the increase in the mean length of axillary shoots (b) during 4 subcultures to the same medium. The basal medium as indicated in the legend of figure 2.

Growth regulator treatments: 1 = without hormones, 2 = 0.89 μM BA, 3 = 0.89 μM BA + 0.29 μM GA_3, 4 = 0.89 μM BA + 2.90 μM GA_3, 5 = 0.89 μM BA + 0.045 μM NAA, 6 = 0.89 μM BA + 0.045 μM NAA + 0.29 μM GA_3, 7 = 0.89 μM BA + 0.045 μM NAA + 2.90 μM GA_3, 8 = 0.89 μM BA + 0.089 μM NAA, 9 = 0.89 μM BA + 0.089 μM NAA + 0.29 μM GA_3, 10 = 0.89 μM BA + 0.089 μM NAA + 2.90 μM GA_3, 11 = 0.89 μM BA + 0.18 μM NAA, 12 = 0.89 μM BA + 0.18 μM NAA + 0.29 μM GA_3, 13 = 0.89 μM BA + 0.18 μM NAA + 2.90 μM GA_3, 14 = 0.89 μM BA + 0.045 μM IBA, 15 = 0.89 μM BA + 0.089 μM IBA, and 16 = 0.89 μM BA + 0.18 μM IBA.

GA_3 was included in the autoclaved media after filter-sterilization.

MICROPROPAGATION OF *PROTEA REPENS*

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Abstract

Multinodal explants (3 — 5 nodes) of *Protea repens* cv. Embers, were established on half-strength Murashige and Skoog medium supplemented with 1 mg.ℓ^{-1} benzyladenine. Treating actively growing shoots on field grown mother plants with 200 mg.ℓ^{-1} benzyladenine, significantly reduced browning and promoted bud sprouting *in vitro*. Treating mother plants with captafol (Difolatan, 1 g.l^{-1}) and iprodione (Rovral flo, 1 ml.ℓ^{-1}), reduced the contamination rate during establishment to 14% compared to 90% of the control. Bud break *in vitro* was increased by the addition of gibberellic acid at 6 mg.ℓ^{-1}. Somatic embryos developed from the base of the shootlet throughout the multiplication stage.

1. Introduction

Propagation by cuttings from single hybrid plants, developed in breeding programs, is slow. Propagation via tissue culture may offer a rapid and commercially viable alternative. The major factors that limit tissue culture of the Proteaceae apparently relates to obtaining sterile explants from field grown plants, phenolic browning of the medium and explant (George and Sherrington, 1984), as well as difficulties in getting axillary buds on explants to sprout (Rugge *et al.*, 1989). Etiolation treatment of the actively growing shoots on the mother plants is apparently helpful to overcome browning and promote sprouting of axillary buds in *Protea obtusifolia* (Watad *et al.*, 1992). In this paper we report on procedures that reduce browning and induce bud break in explants of *P. repens* 'Embers'.

2. Materials and Methods

2.1. Explant preparation

Plant material was obtained from six year old field grown *P. repens* cv. Embers plants. Explants were prepared from distal axillary flushes. To surface sterilize the material, leafy shoots were dipped into a 70% ethanol solution, then agitated for 15 min in a 2% sodium hypochlorite solution with two drops of Agral (surfactant) before rinsing in sterile distilled water. Explants were prepared by sectioning shoots into 20 — 25 mm long pieces with 3 — 5 nodes. The leaves were cut back to 5 mm.

2.2. Medium and cultural conditions

Test tubes (25 x 150 mm) were filled with 10 mℓ of culture solution. The culture solution contained half-strength Murashige and Skoog (1962) macroelements, full strength microelements and vitamins, 3% sucrose, 0.75% agar, and 1 mg.ℓ^{-1}

benzyladenine. The pH was adjusted to 5. Cultures were grown at 25 °C (16 h day) and 22 °C (8 h night). The light source was cool-white fluorescent tubes supplying a light intensity of 45 μ mol.m^{-2}.s^{-1} at culture level.

2.3. Mother plant pre-treatments

2.3.1. Fungicides

Field grown mother plants were pre-treated with different fungicides: (a) iprodione (Rovral flo, 1 $m\ell.l^{-1}$) and captafol (Difolatan, 1 g.ℓ^{-1}), or (b) benomyl (Benlate, 1 g.ℓ^{-1}) and captab (Captan, 1 g.ℓ^{-1}), five and two days prior to picking of the shoots. The control was not treated with fungicides. No rain occurred in the time elapsed between spraying and collection of the explants (experiment was conducted in July). Explants were sterilized and cultured as described and the number of contaminants were noted after 20 days.

2.3.2. Cytokinin

Benzyladenine (BA) at 200 mg.ℓ^{-1} was applied until run-off to the entire mother plant on 6 March. A similar plant was kept as a control. Explants were prepared from actively growing distal axillary flushes (on flower bearing shoots, one month prior to anthesis), on March 28. Thirty explants were prepared for each treatment. After 9 weeks the number of explants which had sprouted and the number of sprouted buds per explant were determined, the browning of the explant was also noted.

2.4. Gibberellic acid treatment *in vitro*

The effects of gibberellic acid (GA_3) on bud sprouting were examined at 0, 3, 6 and 9 mg.ℓ^{-1} in the presence of 1 mg.ℓ^{-1} BA. Mother plants were treated with 200 mg.ℓ^{-1} BA as described above. Thirty explants were prepared for each treatment. After 9 weeks the number of explants which had sprouted and the number of sprouted buds per explant were determined, and the browning of the explant noted.

2.5. Multiplication

Shoot sprouts which developed were excised and subcultured on a multiplication medium containing half-strength MS macroelements, full strength microelements, 3% sucrose, 1% agar, supplemented with 1 mg.ℓ^{-1} BA and 6 mg.ℓ^{-1} GA_3. To encourage axillary bud break a topping method was used in which the top half of the shoots were repeatedly harvested. Observations were made after 8 week subculture intervals.

3. Results and discussion

Pre-treating field grown mother plants with fungicides proved to be very beneficial in reducing the contamination of initial cultures. Using Rovral flo and Difolatan reduced the contamination rate during establishment of the explants to 14%, and 68%

when Benlate and Captan was used, compared to 90% contamination of the control.

Treating actively growing distal axillary shoots on field grown mother plants with 200 mg.ℓ^{-1} benzyladenine, reduced browning and promoted bud sprouting *in vitro* (figure 1). Treating shoots which were not actively growing with 3 consecutive sprays of BA (200 mg.ℓ^{-1}) with 3 weekly intervals elicited a similar response (data not presented). Application of benzyladenine (200 mg.ℓ^{-1}) during summer resulted in the release of axillary buds from correlative inhibition, and subsequently an increase in axillary bud sprouting in *Protea* cvs. Ivy and Carnival (De Swardt, 1989). Spraying, or injecting mother plants with cytokinins to induce bud sprouting have been used to produce cuttings in Cordyline (Maene and Debergh, 1989) and pawpaw (Allan and MacMillan, 1991). Preparation of the mother plant is apparantly of utmost importance in Proteaceae, as in other plants as stressed by Debergh (1987).

Maximum axillary bud sprouting was obtained with 3 — 6 mg.ℓ^{-1} GA_3 (table 2). Increasing the GA_3 concentration to 9 mg.ℓ^{-1} did not result in an increase in the number of sprouted shoots. Various reports have noted the importance of the use of gibberellic acid in establishment and multiplication of Proteaceae (Seelye, 1984; Ben-Jaacov and Jacobs, 1986; Tal *et al.*, 1992; Watad *et al.*, 1992).

Multiplication occurred through: (a) enhanced axillary bud break (figure 1), where new shoots arose from the axillary meristems in the basal portions when the topping method was used. (b) The production of adventitious buds (figure 2), where many shoot primordia formed from the bases of shootlets, but not much elongation occurred. (c) Somatic embryogenesis (figure 3), where somatic embryos formed directly from the base of the shootlets as well as from callus which formed on the bases of the shootlets after two subcultures. The formation of the embryos was usually related to some degree of browning of the explant. Krul and Worley (1977) also observed that developing embryoids of *Vitis* occurred adjacent to areas of necrotic cells. Primary somatic embryos generally failed to mature normally into plantlets and gave rise to successive cycles of embryos. Occasionally some embryos germinated (figure 4) and gave rise to normal plantlets. Rugge *et al.* (1989) reported on aberrant somatic embryos which were observed on the leaves of *Serruria florida* shoots during the proliferation phase. Plant regeneration via somatic embryogenesis has the potential for producing the greatest numbers of plantlets in comparison to axillary bud break and the production of adventitive buds (Thorpe, 1990).

In conclusion, the micropropagation of *P. repens* appears to be possible if actively growing shoots on the mother plants are pre-treated with cytokinins. Somatic embryogenesis could be another possibility for the rapid propagation of Proteaceae.

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Table 1. Effect of benzyladenine pre-treatment on the sprouting of multinodal explants of 'Embers' *in vitro*.

Treatment	% Explants with sprouted buds	No. of sprouted buds/ growing explant	% Browning
Control	0	0	100
Pre-treated (200 mg.ℓ^{-1} BA)	45	1.8	21

Table 2. Effect of gibberellic acid concentration on the sprouting of multinodal explants of 'Embers' *in vitro*.

Gibberellic acid concentration	% Explants with sprouted buds	No. of sprouted buds/growing explant	% Browning
0 mg.ℓ^{-1}	45	1.8	21
3 mg.ℓ^{-1}	65	1.8	31
6 mg.ℓ^{-1}	77	1.6	19
9 mg.ℓ^{-1}	52	1.6	37

Figure 1. Enhanced [illegible] of *Protea* [illegible] *in vitro*.

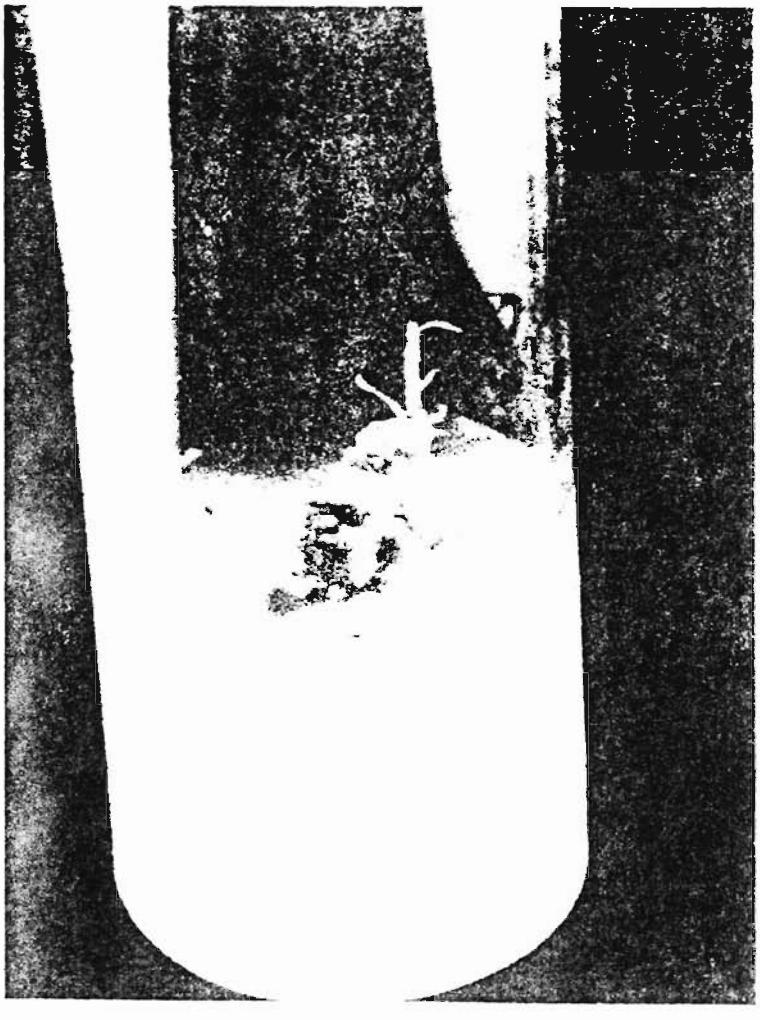

Figure 2. Adventiti[illegible] *Protea* repens *in vitro*.

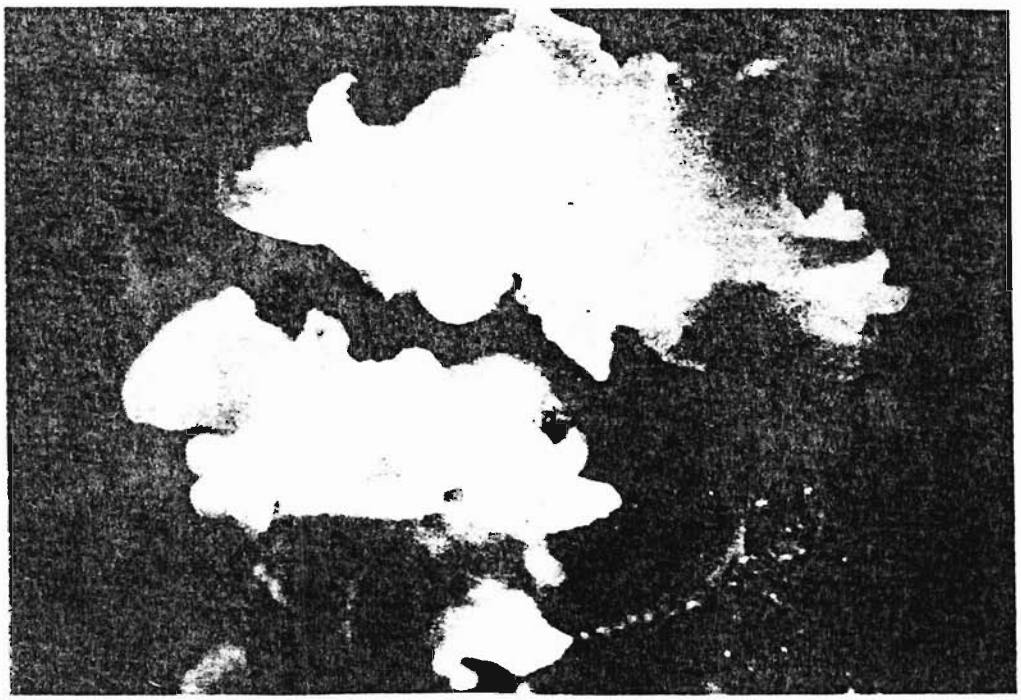

Figure 3. Somatic embryogenesis of *Protea repens in vitro*.

Figure 4. Germinating somatic embryo of *Protea repens in vitro*.

PROTEA PLANT PROTECTION: FROM THE AFRICAN CONTEXT TO THE INTERNATIONAL ARENA

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Abstract

This paper primarily reviews research on pests and diseases of Proteaceae in South Africa. The potential threat that South African pests and diseases pose to the international protea industry is analyzed by considering which pests and diseases have spread from South Africa to the rest of the world. The ability of exotic pests to exploit Proteaceae in South Africa and elsewhere is considered and is shown to be limited. It is concluded that any internationally important new protea pests are likely to originate from South Africa.

Introduction

This paper reviews plant protection research (pest and disease control) on Proteaceae in South Africa, and examines the extent and potential of pests and diseases internationally. South Africa is the source of a substantial number of internationally cultivated Proteaceae species and cultivars. Pests and diseases which have evolved together with African Proteaceae also have their origins in South Africa, with potential to become serious pests in others regions where Proteaceae are cultivated.

The Cape fynbos, the biome in which most of the African Proteaceae occur, is also a renowned centre of plant species diversity (Cowling, 1992). As such, it is of international value to science, providing a natural laboratory for the study of biodiversity and plant-animal interactions. In the process of studying the pests and diseases of the Proteaceae, basic scientific research has been done as well as applied work. This has not only led to applications relevant to the protea industry, but also to the gathering of important ecological data. This review considers entomological and plant pathological research undertaken in South Africa on Proteaceae, and identifies pertinent aspects, both to the protea industry and to the fynbos ecosystem.

Entomology: Basic research

Insect pollination: The role of insects as pollinators of *Protea* spp. and *Leucadendron* spp. has received attention (Coetzee and Giliomee, 1985; Wright *et al.*, 1991; Hattingh and Giliomee, 1988). Some controversy was caused when *P. repens*, originally considered to be bird pollinated, was shown by Coetzee and Giliomee (1985), to be equally effectively pollinated by insects. Suggestions were made by Collins and Rebelo (1987) that bird-pollinated seed would be of genetically higher quality than seeds resulting from insect pollination. Subsequent work (Wright, 1994) has tentatively confirmed this. However, insects have been found to play a role in the pollination of a range of *Protea* species studied (not always as important as birds though), and they should not be ignored as unimportant to the plants (Wright *et al.*, 1991). Insects pollinating *Protea* spp. and *Leucadendron* spp. are generalist flower visitors (Coetzee and Giliomee, 1985; Hattingh and Giliomee, 1988). It is possible that larger beetles (Coleoptera, Scarabaeidae) may be more important pollinators than smaller insects

(Wright, 1990). It is clear that insects inhabiting *Protea* inflorescences have an important ecological role to play, and are not merely pests of cut flowers.

Seed predation: Infructescences (seed heads) of *Protea* spp. are attacked by the larvae of a range of insects (Coleoptera and Lepidoptera). These insects infest the receptacle and bore into the seeds (Myburgh *et al.*, 1975; Coetzee and Giliomee, 1987a). It has been suggested that these insects are ecologically important as a limiting factor in the reproduction of *P. repens* by Coetzee and Giliomee (1987b). Insect seed predation of canopy-stored *Protea* seed banks may be a factor which reduces the potential of these plants to form monospecific stands (Wright, 1993). The lack of single species dominance in fynbos landscapes is characteristic of this flora (Taylor, 1978). Low and variable seed set in *Protea* spp. may be an effective mechanism which allows seeds to escape insect predation (Wright, in press).

Borers attacking *Protea* infructescences are also an important guild of pests of cultivated proteas (Myburgh and Rust, 1975), attacking young shoots and flower buds. Infested infructescences serve as a reservoir where pest numbers can increase (Coetzee and Giliomee, 1987a; Wright, 1990). Studies of the biology of these insects have indicated that orchard sanitation is a practice which should be applied in order to reduce borer incidence (Coetzee *et al.*, 1988).

Herbivory: In the Proteaceae investigated, folivorous insects removed 5 — 22% of the leaf area produced (Coetzee, 1989; Wright and Giliomee, 1992). This damage presents an aesthetic problem for the sale of cut flowers and foliage. Research on physical and mechanical defence mechanisms in these plants have indicated that some of these traits may be of value in a programme selecting for resistance to leaf feeding insects. Trichomes on the young leaves of *Protea magnifica* and *P. laurifolia* were shown to deter leaf feeding (Wright and Giliomee, 1992). Cyanogenic glycosides in *Leucadendron laureolum* appear to play a role in protecting young leaves from insects (Coetzee, 1989a). The general pattern of herbivory for Proteaceae is somewhat unique. More succulent and nutritious young leaves are avoided in favour of older, tougher phenol rich leaves (Coetzee, 1989a; Wright and Giliomee, 1992). This contrasts with herbivory patterns observed in other ecosystems, where young leaves are usually attacked in preference of older leaves (Reichle *et al.*, 1973; Coley, 1980; Lowman, 1984). The older leaves of *Protea* spp. are sub-optimal insect food in terms of low water and protein content and high tannin content (Wright and Giliomee, 1992). However, some of the most important herbivores on *Protea* spp. (*Bostra conspicualis*, Pyralidae, Lepidoptera; *Afroleptops coetzeei*, Curculionidae, Coleoptera), have alimentary tract pH levels which suggest adaptation to a tannin-rich diet (Wright and Giliomee, 1992), which allows them to utilize older leaves in spite of the presence of tannins (phenols).

This pattern of herbivory can be used to facilitate rationalizing pest control measures. As most leaf-feeding damage occurs once leaves have shed their trichomes, pesticide applications are not needed before this stage.

Endophagous insects on Proteaceae: Borers, leaf miners and gall forming insects are rated as some of the most important pests of Proteaceae (Myburgh and Rust, 1975). Borers attack young shoots, buds, stems and roots of Proteaceae, leading to extensive crop losses (Viljoen and Wright, 1991, Wright *et al.*, 1991). Leaf miners cause

scarring on leaves which renders the final product unmarketable, while gall forming insects are a phytosanitary risk.

The control of borers is not easily achieved using conventional control measures. Their characteristically evasive life style (viz. boring into the woody parts of their host), make the use of pesticides unreliable. The timing of pesticide applications has to be exact, and infestations are easily missed. An in-depth study of borer ecology which aims to identify alternative control measures is in progress (Wright, 1992a). To expedite this, an understanding of the ecology of the insects concerned is required. To this end, the biogeography, habitat needs, climatic influences, host plant selection and interactions with natural enemies of borers is being investigated (Wright, 1992a,b). Insects with potential to become new pests are also being identified during the course of this work. Biogeographical data gathered show that some stem and bud borers (*Orophia ammopleura*, Olethreutidae, Lepidoptera; *Erioderes candezei*, Cerambycidae, Coleoptera; *Euderes natalensis*, Curculionidae, Coleoptera) occur not only on Cape Proteaceae but also on more northern species (e.g *P. caffra*). Beetles attacking stems and roots (*Sphenoptera* spp, Buprestidae, Coleoptera) as well as infructescences (various other Coleoptera and Lepidoptera, see Coetzee and Giliomee, 1987a; Wright, 1993) are restricted to the Cape *Protea* species (Wright, unpublished data).

Information relating to the habitat and climatic needs of these borers have indicated that habitat attributes (e.g. plant height and density) are most important, with climate playing a lesser role in ecological terms (Wright, 1993). Climate is no doubt more important as a historic influence on the insects. The role of differential host selection was also found to be important (Wright, 1993). A synthesis of these data will contribute to the development of a integrated control strategy for *Protea* borers. It is envisaged that cultural control measures and borer predictive models will be developed (Wright, 1992b).

Applied entomology

Various short-term studies which have addressed market-related problems have been completed. These include studies on disinsectation of cut flowers, surveys of pest status and registration trials for insecticides.

The presence of insects in cut flowers is one of the most serious limiting factors influencing the South African protea industry. A wide range of species occur in the flowers (Coetzee and Giliomee, 1985), and in many cases, at high population levels (Visser, 1992). Initial attempts to control these insects involved dusting with pesticides (e.g. gamma BHC, Vermeulen *et al.*, 1992). Later efforts included the use of fumigants and injection of pesticides into flowers (Wright and Coetzee, 1992). The use of magnesium phosphide gas combined with dichlorvos ec. or deltamethrin ec. injection was found to give best results (Wright and Coetzee, 1992). Further work showed that the use of a negative pressure fumigation system, based on a forced cooling system, provided excellent insect control using only dichlorvos aerosol (Wright, 1992b). The use of these fumigation techniques, combined with efficient harvesting practices (viz. picking at the earliest, or "soft bud" stage), should enable producers to supply insect-free flowers for export.

Extensive on-farm surveys of the occurrence of pests on proteas in South Africa have yielded data which will contribute to the formulation of predictive models indicating where certain pests are likely to be most injurious (Wright, unpublished data).

These type of data, combined with a quantitative approach to insect ecology, can provide meaningful answers regarding pest control.

Three pesticides (dichlorvos, dimethoate and *Bacillus thuringiensis* var *kurstaki*) have been registered in South Africa for use on proteas (Vermeulen *et al.*, 1992). Pests which have been addressed specifically are the black moth (*Argyroploce* sp., Tortricidae, Lepidoptera), channel leaf miner (*Phyllocnystis* sp., Phyllocnistidae, Lepidoptera), blotch leaf miner (*Protaephagus capensis*, Incurvariidae, Lepidoptera) and pine emperor moth (*Imbrasia cytheria*, Saturniidae, Lepidoptera). More recent attempts to obtain further registrations have been thwarted, as the South African protea industry is too small to capture the attention of the agro-chemical industry.

Plant pathology

A number of diseases have been recorded on Proteaceae in South Africa (Van Wyk, 1973; Van Wyk *et al.*, 1975). The fact that these pathogens occur widely in the native habitat of their host plants indicates that they are indigenous to the region (Von Broembsen, 1986; Knox-Davies *et al.*, 1986, 1987). These pathogens occur throughout the fynbos as well as in other protea production areas, e.g. Natal and Transvaal (Van Wyk, 1973; Benic, pers. comm.).

Leaf diseases

These diseases are caused by pathogens of the classes Ascomyceta and Hyphomyceta (Knox-Davies *et al.*, 1987; Von Boembsen, 1986). Leaf-spot, speck and blotch diseases include the following: *Batcheloromyces (Stegmina) proteae, B. leucadendri, Mycosphaerella proteae, M. jonkershoekensis, Leptosphaeria protearum, Coleroa senniana, Dreschlera dematioidea, Elsinoe* sp., *Phyllachora proteae* and *Vizella interupta*. These fungi cause unsightly marks on the leaves of infected plants, and are a phytosanitary and aesthetic problem. Little is known about the biology, epidemiology and control of these diseases. This is largely because the pathogens all grow extremely slowly under laboratory conditions. The processes and environmental factors associated with leaf infection are poorly understood, so there is little potential for the use of predictive models at present. What does appear to be true is that wet and humid conditions favour the development and spread of these diseases. No fungicides are registered in South Africa for the control of these pathogens (Vermeulen *et al.*, 1992).

Shoot blight, canker and die-back diseases

The pathogens responsible for stem and shoot disorders are *Botryosphaeria dothidea, Dreschlera dematioidea* and *Colletotrichum gloeosporioides*. As in the case of leaf pathogens, little data are available regarding the biology of these organisms. Their control is problematic, since no fungicides are registered for their control on Proteaceae (Vermeulen *et al.*, 1992).

Root and collar rot

Phytophthora cinnamomi (Rands), the pathogen causing root and collar rot in Proteaceae, is a serious limitation in the cultivation of Proteaceae. In South Africa, *P. cinnamomi* occurs in the wild as well as in cultivated areas. Many pristine rivers have been found to harbour and transport the propagules of this pathogen (Von Broembsen and Brits, 1985), suggesting that it is indigenous to the region. Research in South

Africa regarding the control of *P. cinnamomi* has been aimed largely at the development of resistant rootstock cultivars (Von Broembsen and Brits, 1985; Turnbull, 1991). This programme has met with some success, with the development of one partly tolerant cultivar (Van der Merwe *et al.*, 1991).

Internationally, biological control of *P. cinnamomi* on proteas has been attempted with variable success (Turnbull, pers. comm.). using *Pseudomonas cepacia* (strain 65). Further testing must be done to establish whether *Phytophthora* can be controlled under field conditions. Initial results in glasshouses indicate that there is potential for the use of biological control (Turnbull *et al.*, 1989).

Damping-off, seedling blight and decay of cuttings

A wide range of pathogens contribute to disease problems associated with propagation material. Many of these diseases are caused by soil and seed-borne fungi. These include *P. cinnamomi, C. gloeosporioides, Botrytis cinerea, Fusarium oxysporum, Macrophomina phaeseolina, Pythium* spp., and *Rhizoctonia solani* (Benic, 1986). Only one fungicide, fosetyl - Al, is registered against soil pathogens on proteas in South Africa (Knox-Davies *et al.*, 1987; Vermeulen *et al.*, 1992). Forsberg (1993) lists products which are used in other parts of the world.

Witches broom

A number of Proteaceae are susceptible to witches broom. Various studies aimed at elucidating the cause of this condition (e.g. Dorrington, 1987) have been carried out. However, no causal agent has been clearly identified. The current hypothesis is that a mite transmitted, mycoplasma-like organism leads to the proliferation of witches broom on proteas (Knox-Davies *et al.*, 1987).

South African pests and diseases internationally

There are no published records of South African insect pests of proteas that have become established internationally. This is no doubt the result of strict phytosanitary control applied by countries importing plant material.

Most of the South African diseases of Proteaceae have been recorded from other countries (Forsberg, 1993). Those fungal leaf spot diseases occurring in South Africa but not yet recorded (e.g. *Batcheloromyces, Leptosphaeria*) may become troublesome should they be imported and allowed to occur under favourable climatic conditions.

Exotic pests on Proteaceae in South Africa

The occurrence of pests from other counties or crops on proteas in South Africa should provide an indication as to whether proteas grown overseas are likely to be susceptible to a wide range of pests. Exotic pests recorded on proteas in South Africa are: boll worm (*Heliothus armigera*, Noctuidae, Lepidoptera), carnation worm (*Epichoristodes acerbella*, Tortricidae, Lepidoptera) and lucerne butterfly (*Colias electo*, Pyralidae, Lepidoptera) (Viljoen and Wright, 1991; Wright, unpublished data). This is an astoundingly short list, considering that numerous exotic pests occur on various crops in South Africa (Annecke and Moran, 1982). it is possible that the generally poor quality of proteas as a source of insect nutrition (Wright and Giliomee, 1992) may have precluded their becoming exploited by pests of other crops.

Other pests and diseases internationally

Published records of protea pests in countries other than South Africa are limited to the USA and Australia (Anon., 1993; Coetzee, 1989b). In the USA, Argentine ants (*Iridomyrmex humilis*, Formicidae, Hymenoptera), Fuller rose beetles and thrips (Thysanoptera) have achieved pest status (Coetzee, 1989b). In Australia leaf miners, borers, leaf feeders and scale insects have been reported to be injurious on cultivated proteas (Anon., 1993). Scale insects, leaf rollers and some mites are reputed to be problematic in New Zealand (Turnbull, pers. comm.). It would therefore appear that countries without indigenous Proteaceae (e.g. the USA), do not have any of the most injurious guilds of insect pests noted in countries with Proteaceae.

Proteaceae diseases not recorded in South Africa, but indeed elsewhere, are bacterial leaf spot, *Armillaria* sp. and *Verticillium* sp. (Forsberg, 1993). *Verticillium dahliae* Kleb. has been shown to be the pathogen causing wilt of *Leucospermum cordifolium* in California (Koike, 1991).

Establishment of South African Pests internationally

South Africa is the source of a great diversity of protea cultivars, and is likely to remain the main bank for the industry. However, the worst possible pests attacking these Proteaceae also originate from South Africa. It is therefore pertinent to the international protea growing community to establish which South African pests are most likely to establish in the various protea growing countries and in what areas. The use of climate matching of areas has been used the past by researchers planning introductions of bio-control agents to a new country. A computer programme, CLIMEX, has been developed in Australia to facilitate this process. The programme compares a source locality (e.g. Cape Town, South Africa) to a country of potential insect introduction (e.g. Australia) in terms of climatic similarity. Maps are produced (e.g. figure 1) with dots identifying areas exhibiting climates of a requested similarity (e.g. 60%) and higher. The greater the climatic similarity, the greater the probability that pests will establish in the introduction area.

To identify areas in the USA, Australia and New Zealand which would be hospitable to South African pests, two of the most important borers of *Protea* spp. in South Africa with reasonably well known climatic preferences were chosen for climate matching. These are the stem and bud borers *Orophia ammopleura* (Oethreutidae, Lepidoptera) and *Argyroploce* (Tortricidae, Lepidoptera). *Orophia* is more prevalent in cooler areas, and *Argyroploce* prefers warmer sites (viz., further east in the Cape). Climatic matching for these species showed that *Orophia* is likely to become established in protea growing areas in California (figure 1), Australia (figure 2) and New Zealand. *Argyroploce* is less likely to establish in the USA growing areas, but Australia and New Zealand (figure 3) should be prone to invasion. These results indicate that care should be taken that these pests do not reach these areas. Strict phytosanitary control in these countries should be applied to ensure that no accidental introductions have occurred.

Conclusions

A wide range of topics regarding insect-plant interactions and insect control on Proteaceae have been researched in South Africa. These studies have shown that insects are not only pests of proteas, but also play an ecologically important role in fynbos. They are also an important component of the faunal biodiversity of the region.

Proteaceae diseases have been less extensively studied, and remain an enigmatic and important problem in South Africa and internationally. Biological and epidemiological studies are of particular importance to the industry.

The present situation where only a few exotic pests attack proteas in South Africa, and few indigenous insects in other parts of the world, suggests that any important new pests internationally will come from South Africa (and possible Australia). Climate matching shows that some of the most important South African pests are likely to be able to establish in countries currently growing proteas. However, South Africa is also likely to be the source of any plant resistance to these pests. This underscores the importance of the natural gene pool endemic to the Cape Fynbos, and the critical need to preserve and nurture it for future use.

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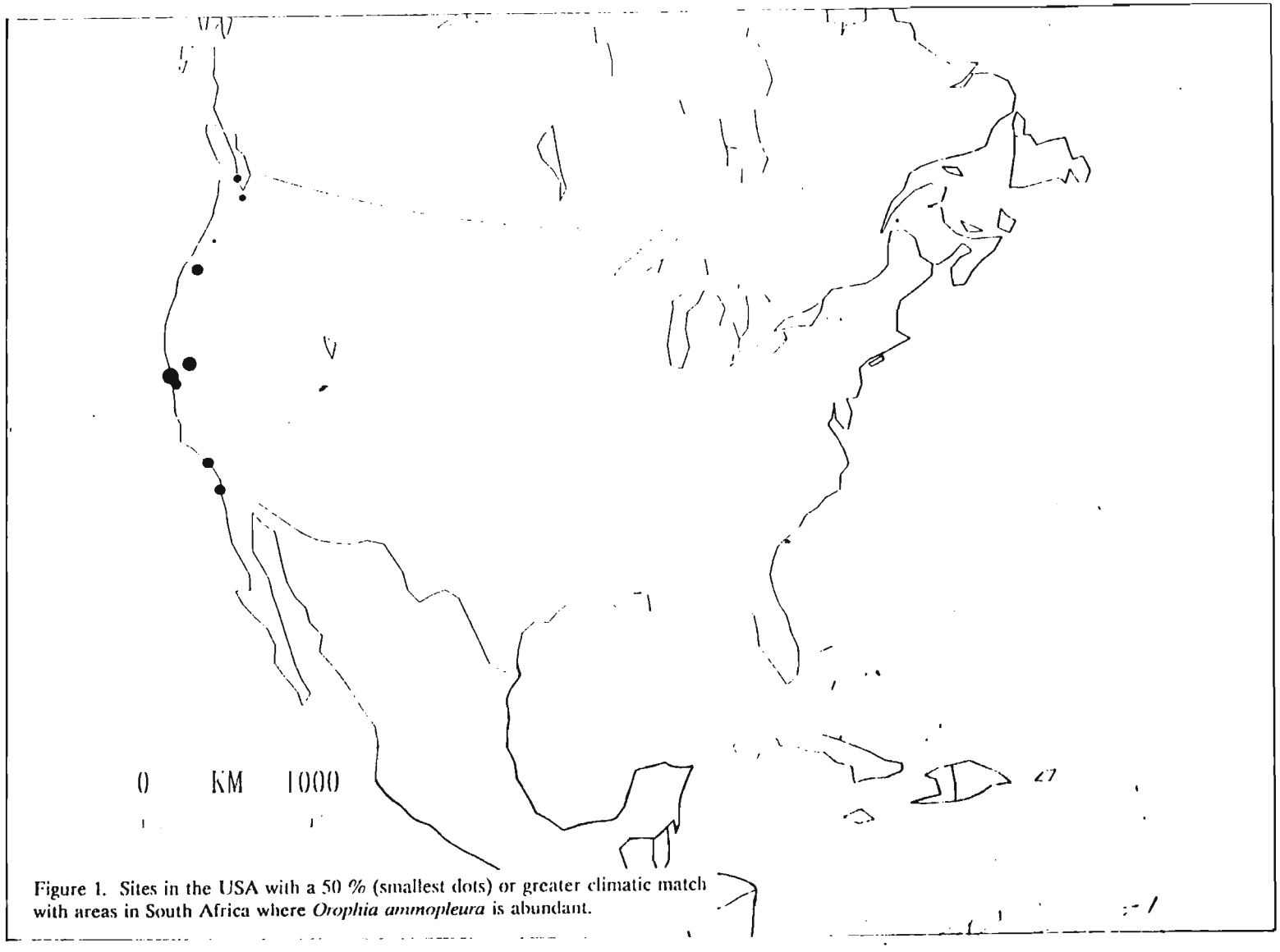


Figure 1. Sites in the USA with a 50 % (smallest dots) or greater climatic match with areas in South Africa where *Orophia ammopleura* is abundant.

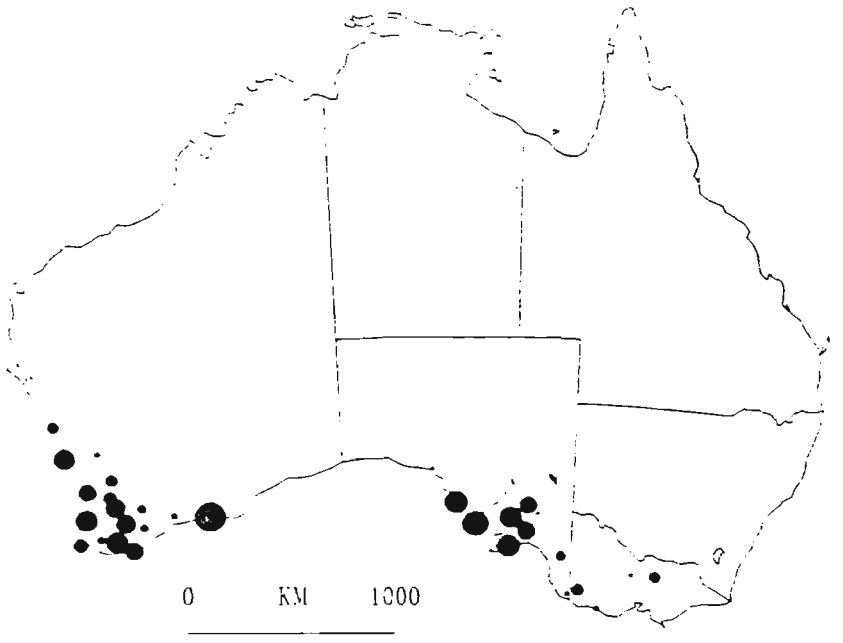
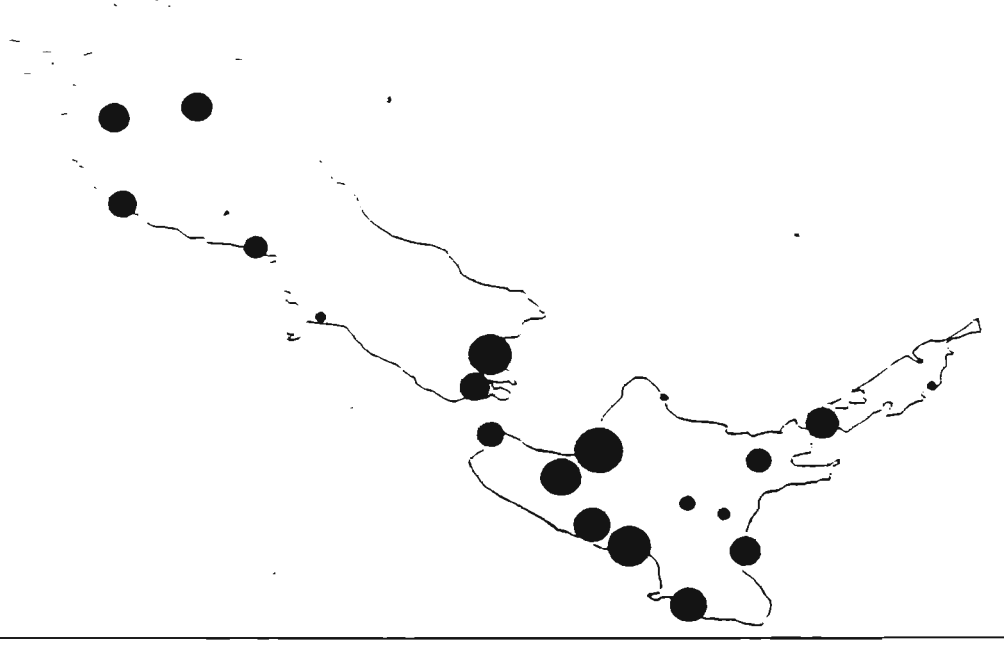


Figure 2. Sites in Australia with a 60 % (smallest dots) or greater climatic match with areas in South Africa where *Orophia ammopleura* is abundant.

Fugure 3. Sites in new Zealand with a 50 % (smallest dots) or greater climatic match with areas in South Africa where *Argyroploce* sp. is abundant.



FIELD STUDIES ON THE EFFECTIVENESS OF PHOSPHONATE SUPPRESSION OF *PHYTOPHTHORA* ROOT ROT IN PROTEAS

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Abstract

Phosphonate suppression of root rot in proteas, caused by *Phytophthora cinnamomi*, was tested in field trials over a two-year period. Effectiveness of chemical control varied, dependent upon plant species, site topography, soil moisture content and soil temperature.

Phosphonate, applied as a foliar spray at 1.2 g a.i.ℓ^{-1} at 6 weekly intervals, resulted in 100% plant survival (*cf* 19% in unsprayed control plants) in *Leucadendron* 'Sylvan Red' and 75% survival in *Leucospermum* 'Firewheel' (*cf* 44% in unsprayed plants). Under site conditions conducive to high disease pressure, increased spraying intensity (3 weekly, 2.4 g a.i. ℓ^{-1}) did not prevent infection (81% of plants) and death (50%) of *Leucospermum* 'Firewheel' by *P. cinnamomi*.

1. Introduction

The root and collar-rot fungus, *P. cinnamomi* (Rands) is a major limitation to the commercial cultivation of proteas. This pathogen is present in both cultivated and uncultivated soils and also occurs in ground water, dams and rivers. *P. cinnamomi* has a very wide host range, infecting both woody (e.g. avocados) and non-woody (e.g. many ornamentals) species (Forsberg, 1989).

Methods of disease control include use of tolerant rootstocks, biological (e.g. mulches) and chemical agents. Success in disease control varies from species to species. In the avocado industry a high level of success has been obtained using a combination of biological and chemical control (Coffey, 1985). Similar strategies have not been very successful when applied to Proteaceae.

Phytophthora tolerant rootstocks are being developed for use with *Protea* and *Leucadendron* species (Turnbull, 1991) but may not be commercially available for some years. These studies found no suitable rootstock for use with *Leucospermum* species.

Biological control using a bacterium antagonistic to *Phytophthora* has been found to be effective in the nursery (potted protea plants) environment (Turnbull *et al.*, 1989), but these results were not sustainable in the field (Turnbull, 1992).

Success with chemical control of *Phytophthora* in proteas has also been variable, both between species, sites and years. Current chemicals being used to control *Phytophthora cinnamomi* do not appear to be capable of curative action *once infection has occurred*, particularly where disease pressure is high (Marks and Smith, 1988; 1990). Disease activity is highly dependent upon soil moisture status and temperature. Current spraying practises in Australia favour 4 — 6 weekly applications of phosphonate (1.0 g a.i.ℓ^{-1}) during the summer months (October to March), when the

disease is most active. However in a non-irrigated crop this may result in wastage of chemical when summers are dry, limiting disease activity. Conversely, failure to spray during unseasonal wet periods (e.g. autumn) can result in crop losses as high as 40% (McCredie *et al.*, 1985). In addition, for highly susceptible species, the spraying interval may be too great when disease pressure is high.

The mode of action of phosphonate is not known. Guest and Bompeix (1990) have suggested that phosphonate may act directly to stimulate the hosts defence mechanism, or indirectly, by inducing changes in pathogen metabolism which in turn elicit a rapid defence response in the plant. Critical tissue levels of phosphonate required to maintain an adequate defence response are not known. Studies in avocadoes (Pegg *et al.*, 1990) have shown that accumulation of phosphonate in plant tissue is influenced by the pattern of plant growth. Phosphonate applied as a foliar spray to plants during active shoot growth (flushing) was preferentially transported to the shoots, rather than to the infection court, the roots. It is not known if this relationship between shoot flushing and phosphonate accumulation occurs in proteas. In one of the following trials, attempts were made to match spraying schedules to plant growth activity and to level of disease pressure (i.e. to soil moisture and temperature status), in *Leucospermum* 'Firewheel'.

The following studies investigated the potential of phosphonate to reduce plant losses due to infection by *P. cinnamomi* in two species of protea.

2. Materials and methods

The trials were situated on a well-drained red earth on a commercial protea farm at Caboolture, Queensland. This area experiences mean monthly temperatures varying from 7 to 29 °C and an annual rainfall of 1270 mm, 70% of which falls between the summer months of October and March. The area was known to be infested with *P. cinnamomi*, resulting in 80% loss of *Leucospermum* 'Firewheel' during 1989, and subsequently left fallow.

Two trials were conducted over consecutive years during 1990 — 1992. Each trial site was mounded, approximately 5 m in width (east to west), with a decline at both the south and north ends of the mounds. Plants were established across each mound (E-W), with treatments replicated down the N-S or S-N slope. Both trials employed 4 replications of each treatment, with 4 plants per treatment plot at a density of 1 plant.m^{-2}, in a randomised complete block design.

The site was subjected to the normal irrigation and weed control practices, as carried out by the site owner.

The test species used were *Leucadendron* 'Silvan Red' (Trial 1) and *Leucospermum* 'Firewheel' (Trials 1 and 2), both highly susceptible to *P. cinnamomi* (Turnbull, 1991). Plants were produced from cuttings under disease free conditions in a glasshouse and field planted from 15 cm pots at 3 (Trial 2) to 6 (Trial 1) months of age. For both trials, plant mortality was recorded on a 3-weekly basis, with dead plants being root sampled to determine the presence of *P. cinnamomi*. On completion of Trial 2 all surviving plants were removed and tested for infection by *P. cinnamomi*. Soil temperature and moisture content at 15 cm depth were recorded daily between 8 — 9 am at the trial site.

2.1. Trial 1

Separate planting sites were used for each test species, *Leucadendron* 'Silvan Red' and *Leucospermum* 'Firewheel'.

Two fungicides were used, phosphorus acid or phosphonate, (Foli-R-Fos 200) and furalaxyl (Fongarid), with no fungicide as the comparison treatment. Foli-R-Fos 200 was applied as a foliar spray (1.2 g a.i.ℓ^{-1}), at planting in July 1990 and thereafter at 6-weekly intervals. In a separate treatment, Fongarid was applied as a soil drench (400 mℓ plant^{-1}), at planting and again in January 1991 (when disease pressure was considered to be high) in addition to a 6-weekly foliar application of Foli-R-Fos 200. For the area treated around each plant, the soil drench rate approximately equated to 4 g Fongarid.m^{-2}.

2.2 Trial 2

Phosphonate was applied as a foliar spray using Foli-R-Fos 200 and Foli-R-Fos 400 at 1.2 and 2.4 g a.i.ℓ^{-1}, respectively. Spray regimes tested were no spray (control), spraying at 3-weekly intervals with Foli-R-Fos 200 (FRF 200), or 6-weekly with Foli-R-Fos 400 (FRF 400) and spraying with Foli-R-Fos 200 (SF 200) or Foli-R-Fos 400 (SF 400) at 3-weekly intervals or greater, dependent upon the rate of new shoot production occurring in the previous spray treatments.

Three weeks prior to field planting on 14 October, 1991, Foli-R-Fos treatment plants were sprayed with the appropriate concentration of phosphonate. These plants were also soil drenched with 500 mℓ of Foli-R-Fos 200 at planting, while the control plants received 500 mℓ of water.

Shoot number per plant was recorded at 3-weekly intervals in the control, FRF 200 and FRF 400 treatments. This treatment was included in order to assess whether phosphonate applied at more than twice the recommended rate (FRF 400) was inhibitory to shoot growth. It also provided the basis for determining the frequency of spraying in the SF 200 and SF 400 treatments.

3. Results

3.1. Trial 1

3.1.1. *Leucadendron* 'Silvan Red' *uc*

Plant deaths were first recorded in the unsprayed plots in October, mortality rates increasing with soil temperature to January and February, then declining, with little further change from April to the end of the trial in July (figure 1). There was no relationship between the pattern of plant mortality and site topography ($P > 0.05$). At the end of the 12 month trial period, one of the 16 control (unsprayed) plants remained. All dead plants tested were infected with *P. cinnamomi*.

Six weekly spraying with Foli-R-Fos 200 resulted in no plant deaths occurring during the trial period, with no advantage ($p< 0.05$) in soil drenching with Fongarid, where 15 of the 16 treated plants survived. Survival of sprayed plants (97%) was significantly greater ($P < 0.01$) than that of unsprayed (19%) plants.

3.1.2 *Leucospermum* 'Firewheel'

The pattern of plant deaths was similar to that recorded for 'Silvan Red', with the first plant death occurring in November, mortality rates increasing and declining with the seasonal change in soil temperature (figure 1). For this cultivar however, plant survival was determined more by site topography than by treatment applied (table 1). In the untreated control plots, plant mortality varied from 0 to 25%, in blocks 1 and 2 at the high end of the site, to 100% in blocks 3 and 4 at the low end of the site, with an overall mean of 56%.

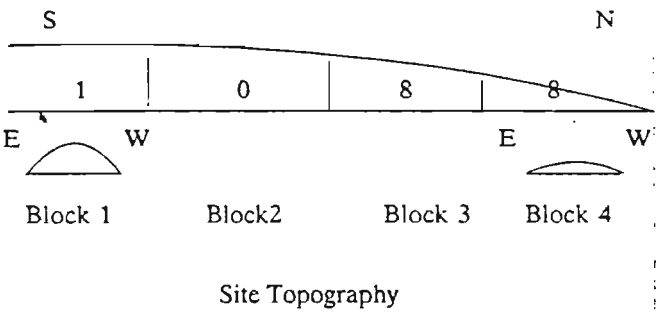
Six-weekly application of Foli-R-Fos 200 reduced plant mortality to 19%, with no significant ($P > 0.05$) change in protection being afforded by the addition of the Fongarid treatment, where 31% of plants died.

Over all treatments, plant survival declined with slope from 92% to 100% to 33%, with deaths of 1, 0, 8 and 8 plants (out of 12), in blocks 1, 2 ,3 and 4 respectively (table 1). Significantly ($P < 0.01$) more plants survived in blocks 1 and 2 at the high end of the site compared to those in blocks 3 and 4 at the low end of the site.

Table 1. Effect of site topography on response of *Leucospermum* 'Firewheel' to chemical treatments to control *Phytophthora cinnamomi*.

(Data are number of plant deaths at 9.7.91, out of 4 planted on 8/7/90).

Treatment	Block 1	Block 2	Block 3	Block 4
Control	1	0	4	4
Foli-R-Fos 200 foliar spray (PHA)	0	0	2	1
Fongarid soil drench (F) + PHA	0	0	2	3
Block Tot	1	0	8	8



3.2. Trial 2

3.2.1. *Leucospermum* 'Firewheel'

Shoot appearance was slow for the first 60 days, increasing gradually, with rapid gains in shoot number after 82 (Control, FRF 400) to 100 (FRF 200) days after planting (figure 2). Analysis of day 120 data showed no difference ($P < 0.05$) in mean shoot number per plant between the FRF 200 and FRF 400 treatments. Wide variability in shoot number was recorded between plants within treatments. This, in conjunction with decreasing sample size as plants died, contributed to the apparent decline in shoot number towards the end of the trial (figure 2).

Spraying of SF 200 and SF 400 treatments was based on shoot numbers recorded from FRF 200 and FRF 400 plants. This resulted in 3-weekly spraying throughout the trial period with the exception of day 190 for SF 200 plants and days 19, 82 and 190 for SF 400 plants.

Plant mortality was first recorded in the Control treatment, in January, 82 days after planting (figure 2). By day 100, significantly ($P < 0.05$) more plants had died in the control than in any of the phosphonate spray treatments. A rapid increase in plant death occurred in all treatments after day 122. This coincided with a period of high rainfall (310 mm fell in 2 days), during February and March. By day 148, differences in plant survival among the phosphonate treatments had emerged, with significantly ($P < 0.05$) more deaths in SF 200 than in either SF400 or FRF 400 treatments. By day 169, mortality levels in the SF 200 plots were similar ($P > 0.05$) to that of the unsprayed Control, being greater than that in SF 400, FRF 200 and FRF 400 treatments, which did not differ significantly ($P > 0.05$). However, subsequent additional plant deaths in these latter treatments resulted in their being no significant ($P > 0.05$) difference between any treatment by day 190. Few plant deaths occurred after day 190 (April).

Site topography had no effect ($P > 0.5$) on the pattern of plant mortality.

P. cinnamomi was isolated from 52 of the 54 plants which died during the trial. Stem girdling was apparent in the dead plants infected with *Phytophthora*. Of the 26 live plants remaining at the end of the trial, *P. cinnamomi* was isolated from the roots of 23 plants (table 2).

Soil temperatures at 15 cm depth, at 8 — 9 am. increased from (3-weekly means) 24 °C in October to 29 °C in January and declined to 19 °C in May. Soil moisture tension varied widely between the 4 tensiometers at the trial site. Overall, soil moisture tension declined, (moisture increased) with time from planting, related to the incidence of rainfall (figure 2).

Table 2. Effect of phosphonate on plant infection and mortality due to *Phytophthora cinnamomi* in field grown plants of *Leucospermum* 'Firewheel' over the period 16.10.91 – 1.6.92.

Treatments	Plant status at the end of the trial (Number of plants out of 16)			
	Dead plants	Dead plants infected with *P. cinnamomi*	Live plants	Live plants infected with *P. cinnamomi*
Control	15	14	1	1
FRF 200	11	10	5	5
FRF 400	8	8	8	5
SF 200	14	14	2	2
SF 400	6	6	10	10
Total (out of 80)	54	52	26	23

4. Discussion

4.1. Factors influencing the level of disease pressure

4.1.1. Soil temperature

Temperature is known to play a significant role in the development of diseases caused by *P. cinnamomi* (Zentmyer, 1981). Increased activity of the pathogen may result in higher levels of root infection taking place; it may also increase the progression of infection within the roots, once infection has occurred. In *Phytophthora* susceptible *Eucalyptus marginata* (jarrah), the extent of hyphal extension of *P. cinnamomi* in the plants roots has been shown to be temperature dependent (Grant and Byrt, 1984). At soil temperatures of 14 °C, infection of jarrah occurred, but extension of the pathogen within the roots was contained, whereas disease progression (hyphal extension), continued at soil temperatures of 24 and 28 °C. In the protea species used in the field trial, plant deaths were first recorded (unsprayed plants) when mean soil temperatures at 15 cm reached 24 °C in November, mortality rates declining when soil temperature fell below 24 °C in April. Plant death appeared to be due to stem girdling; this was evident in most plants tested, even where apparently healthy roots were present. This would suggest that the dominant effect of soil temperature was to alter the rate of progression of *P. cinnamomi* within the roots of plants *already* infected with the pathogen. If so, this response means that for maximum efficacy, the applied treatment should be capable of preventing infection occurring.

4.1.2. Soil drainage

The response to site topography in 'Firewheel' in the first trial infers that the effectiveness of spraying was probably related to disease pressure, if higher at the lower (more poorly drained) end of the site. In this situation, foliar spraying with Foli-R-Fos 200 at 6 weekly intervals was inadequate to prevent plant infection by *P. cinnamomi*. Soil sampling was undertaken in an attempt to quantify site variation in drainage characteristics (bulk density, moisture content) and levels of *P. cinnamomi*. However, the results were too variable to be conclusive. In the second trial, due to heavy rainfall, the soil over the entire site became saturated, eliminating effects of site topography on soil drainage.

4.2. Factors influencing plant response

The response to site topography of 'Firewheel' in the first trial raises the question as to the absence of a similar response in 'Silvan Red', planted on a site with similar topography. Although protea species are known to differ in their tolerance to infection by *P. cinnamomi* when exposed to similar levels of the pathogen, for the two species tested, 'Silvan Red' was considered less tolerant than 'Firewheel' (Turnbull, 1991). This was demonstrated in the field trial, where mortality of the untreated plants was greater for 'Silvan Red' (81%) than for 'Firewheel' (56%). Levels of *P. cinnamomi* may have differed between sites, or conversely, difference in species response to the chemical control agent may have been the dominant factor determining plant survival. The mode of disease control by phosphonate is not understood, nor are the critical levels required in plant tissue to maintain effective disease control, known. A noticeable difference between the two test species used was in the rate of growth following planting. 'Silvan Red' had a relatively slow rate of growth compared to that of 'Firewheel', which continued to 'shoot flush' throughout the test period. In avocadoes, work by Pegg *et. al.* (1989) has shown that when applied during shoot flushing, phosphonate tended to accumulate in the shoots, with little export to the roots. Maximum root accumulation occurred when stem injections of phosphonate were applied after shoot flushing had ceased. If continuous 'shoot flushing' in *Leucospermum* 'Firewheel' resulted, over time, in inadequate tissue concentrations of phosphonate in the roots, the site of infection by *P. cinnamomi*, then frequency of foliar application of Foli-R-Fos 200 may have been the cause of the failure of the chemical control treatment in 'Firewheel' during the first trial. If so, this problem might have been expected to have been solved by the increased intensity of spraying that occurred in the second trial. However, 3-weekly spraying with Foli-F-Fos 200 at more than double the recommended concentration (SF 400 treatment) was unable to prevent plant infection and death. That the FRF 400 and SF 400 treatments had more (although not significantly so), live plants remaining at the end of the trial than the FRF 200 and SF 200 treatments, infers some influence of tissue concentration of phosphonate on plant survival. Isolation of *P. cinnamomi* from 23 of the 26 live plants remaining at the end of the trial suggests that the effect of spraying with phosphonate was to limit rather than prevent disease development.

In the first 100 days after planting 'Firewheel', more than 50% of the unsprayed (control) plants died. During this time only one death occurred among the

plants sprayed with phosphonate. However, after day 122, phosphonate protection appeared to break down, as plant deaths increased in the phosphonate treatments. This response coincided with a period of warm temperatures and very high rainfall (Wilcocks, 1991) in February (expected rainfall 198 mm, actual 408 mm) and March (expected 187 mm, actual 334 mm). These conditions would be conducive to pathogen growth (Zentmyer, 1980,) resulting in an increase in disease pressure at the site. Phosphonate spray schedules that were adequate to control *P. cinnamomi* under the initial disease pressure at the site were inadequate under these conditions.

More work needs to be undertaken to determine the fate of phosphonate in the plant with time after application and the minimum tissue concentration required, ideally, to prevent infection of the plant by the pathogen.

5. Conclusions

Phosphonate applied as a foliar spray at 3-weekly intervals to *Leucospermum* 'Firewheel' and at 6-weekly intervals to *Leucadendron* 'Sylvan Red', is capable of reducing plant mortality due to infection by *Phytophthora cinnamomi*. However, level of disease control will vary, dependent upon disease pressure.

Acknowledgements

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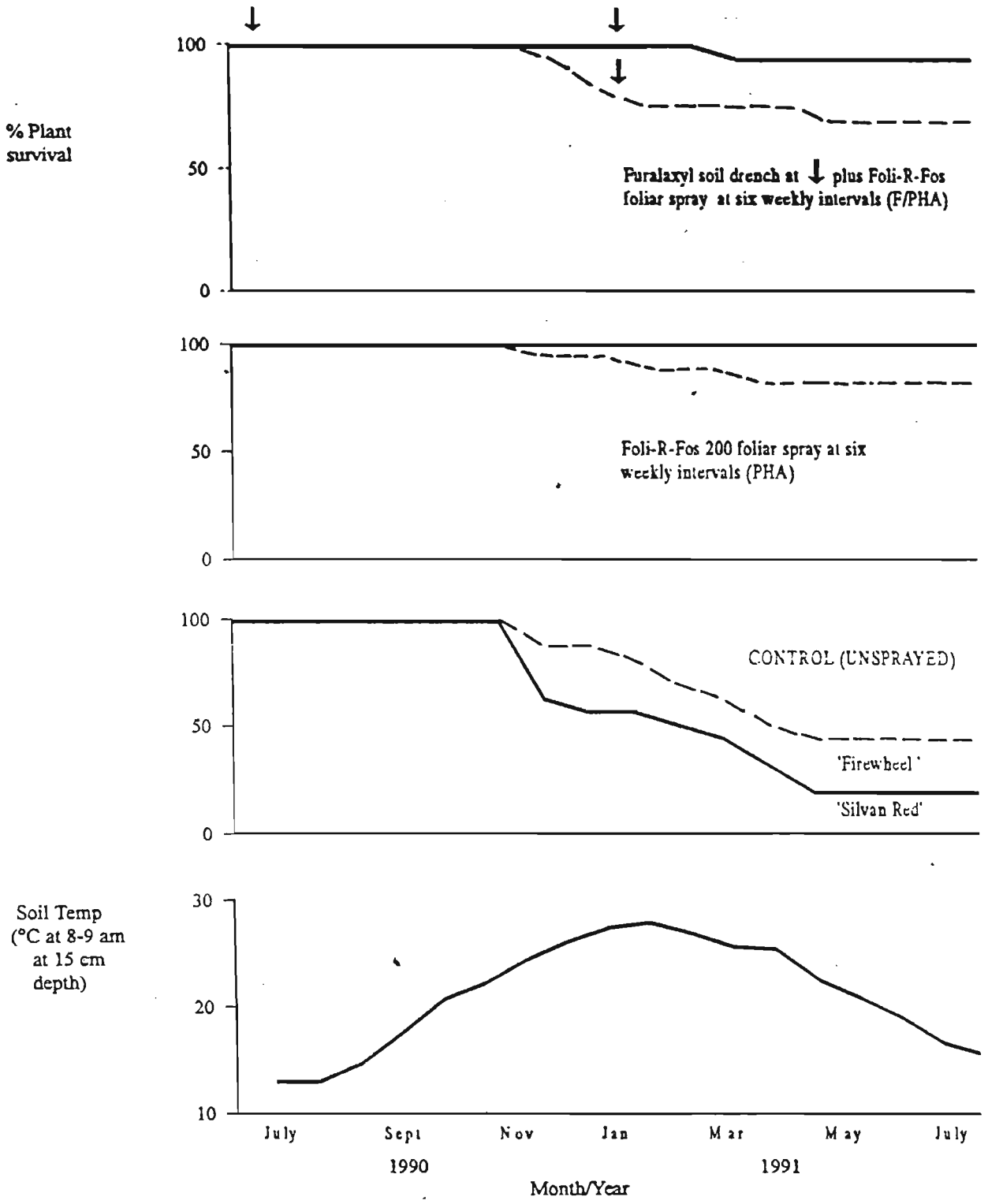

Figure 1. Influence of soil temperature and chemical (Phosphonate, Furalaxyl) treatments on the survival of *Leucospermum* 'Firewheel' and *Leucadendron* 'Silvan Red' in S.E. Queensland (28°S).

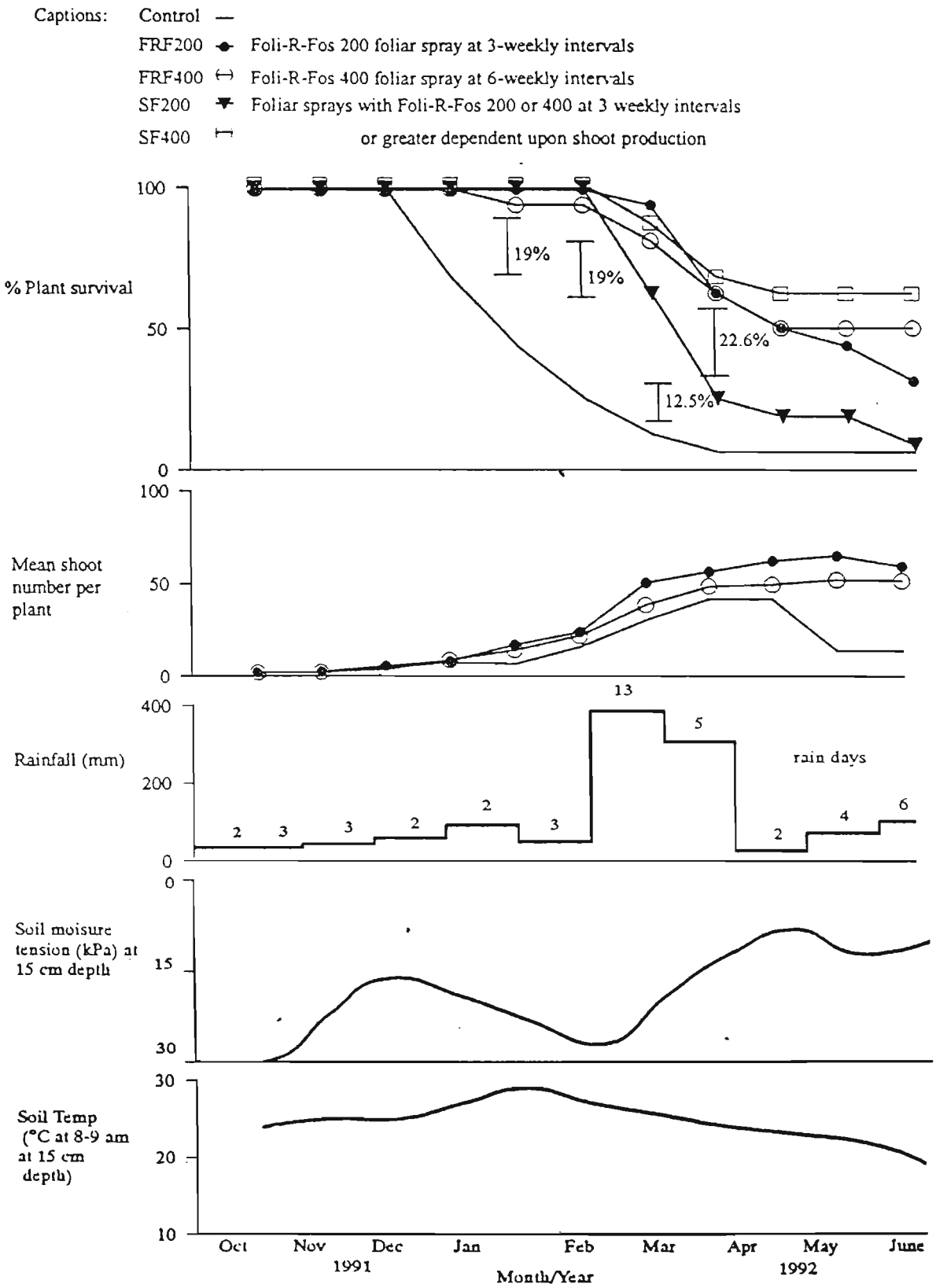

Figure 2. Influence of soil temperature, soil moisture and phosphonate (FRF, SF) spraying regimes on shoot production and survival of *Leucospermum* 'Firewheel' plants growing in *Phytophthora cinnamomi* infested soil SE Queensland (28 °S).

INTEGRATED PEST MANAGEMENT - CONCEPTS AND POTENTIAL FOR THE CONTROL OF BORERS ON PROTEAS

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Abstract

Basic integrated pest management concepts are outlined. Where possible, these are related to protea pest control, and the potential value of such practices is elucidated. A case study considering the control of a stem borer on a *Leucadendron* cultivar is explained, highlighting the value of a range of alternative control measures.

1. Introduction

Integrated pest management (IPM) is a pest management system which utilizes all suitable techniques in as compatible a manner as possible, to maintain pest population levels below those causing economic injury (Dent, 1991). Environmental factors and pest population dynamics must be considered, and the role of beneficial insects as biocontrol agents must not be ignored. The emphasis is on pest management, not on pest eradication. The main reasons for applying such an approach are: to reduce chemical use/abuse by only using pesticides when justified; to avoid causing a build-up of pesticide resistance; to promote worker and consumer health by reducing their exposure to harmful chemicals.

As borers attacking protea crops can usually not be controlled by conventional methods, an IPM approach is required (e.g. Viljoen and Wright, 1991). IPM techniques include using a monitoring system, applying thresholds, encouraging biological control, cultural control, physical control and chemical control.

2. IPM concepts

2.1. Monitoring

Monitoring entails a regular inspection of plants for pests. This results in the farmer being aware of what numbers of pests are present at any time. Plant growth stages which may be particularly susceptible to attack will also be detected. It is important that each farmer is aware of what the situation is on his plants. Pest populations are very variable and population levels vary from site to site, even on a relatively fine geographical scale. On proteas, monitoring for borers should be carried out by inspecting shoots and flower buds for the presence of borer eggs or signs of young borers. As far as borers are concerned, it is most important to monitor shoots/flushes which are going to bear flowers. Monitoring for their presence will result in the farmer becoming aware of them timeously, and in this way pesticide applications can be carefully targeted. Wasteful early, or disastrously late pesticide applications can thus be avoided.

2.2. Thresholds:

Thresholds are defined as levels of pest damage which warrant the use of plant protection measures. Three types of thresholds can be recognized: (1) Economic damage (ED) threshold; (2) Economic injury level (EIL) threshold; and(3) Economic threshold (ET) (Kumar, 1984). The threshold for ED is defined as the amount of damage that justifies economic control. The ED is caused by a population of insects which exceeds the EIL. The ET is applied at a point before insect numbers reach the EIL — i.e. the ED level is not reached. Monitoring is of cardinal importance in discovering when the EIL is reached.

The simplest form of threshold which may be applied is deciding to apply pesticides only when the costs of doing so is justified by level of crop loss. Crop loss to borers in proteas for example, is easily determined. Each bud/stem which is attacked may be considered lost. Monitoring for these pests is therefore simple, as is determining financial loss per-hectare of plants. Spraying costs are also easily calculated. A hypothetical illustration of the application of a threshold at the ED level is shown in figure 1. Percentage infestation in this case could be estimated as number of buds/stems in a sample of 100 stems which is infested with eggs of bud/stem borers. The cost of crop loss is simply a percentage of the estimated yield per hectare lost to borers. If the cost of control is superimposed on the graph, it becomes clear that at about 5% infestation it is economically viable to apply a suitable insecticide. At a lower infestation, the cost of insect control exceeds the value of the crop lost and financial losses are still incurred.

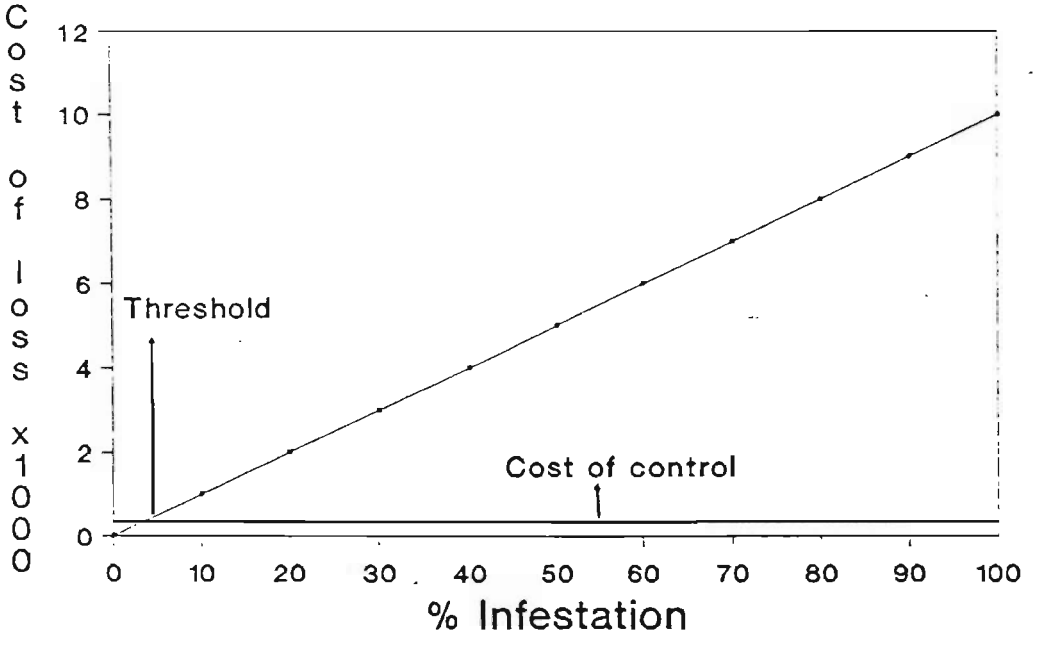

Figure 1. A hypothetical representation of the application of an economic damage threshold for protea borers.

2.3. Encouraging biological control

In natural ecosystems, insects numbers are usually kept low by natural enemies, i.e. predators and parasites. When plants are grown in monocultures and pesticides are applied, this natural balance is upset and pest outbreaks can occur. Encouraging biological control in protea orchards can essentially be reduced to conserving natural enemies of pests. It is not presently possible to rely on biological control for protea borers, so pesticides must still be used. This means that natural enemies as well as pests will be destroyed by pesticides. Judicious use of insecticides can contribute to conserving predators and parasites. The most effective means of achieving a situation where insecticides are used judiciously, is to use monitoring and even a rudimentary form of threshold. The use of "softer" insecticides is also of great value. This is not easily achieved for proteas, as few products are registered for use on them, at least in South Africa.

2.4. Cultural and physical control

Cultural control of pests involves using agricultural practices which make plants within the agro-ecosystem less susceptible to attack by pests. Old and traditional control methods are often used. An important aspect is ensuring that the plants are in a good condition. Healthy plants often have higher tolerance levels for pests. Other commonly used cultural practices such as polycropping and intercropping have not been well researched for use on proteas. The development of predictive models aimed at predicting borer incidence on various *Protea* spp. and cultivars is under way (Wright, unpublished). If it is possible to predict that certain plant varieties are more susceptible under certain conditions (climatic or habitat), careful choices of what varieties to cultivate in which localities can be made. Initial indications are that habitat manipulation may influence borer incidence (Wright, 1993). Climate also plays a role (Wright, 1993), and in conjunction with correct planting choices, borer problems may be reduced.

Physical control involves removing pest insects manually or by means of traps. Orchard sanitation is a valuable physical control practice, and should be used to ensure that any borer-infested plant material in orchards is removed and destroyed. This includes infested stems and seed heads left on plants after flower harvesting (Coetzee *et al.*, 1988). The use of insect traps (e.g. light traps) may have some potential for the control of the adults of some borers (e.g. black moth, *Argyroplce* sp, protea speckled borer, *Orophia ammopleura*). This approach is already used in citrus production in some cases (D.G. Malan, pers. comm.).

2.5. Chemical control

The use of pesticides should be based on monitoring data and damage thresholds as explained above. Over-frequent pesticide application can lead to the build-up of pesticide resistance and causes the destruction of natural enemies of pests. Any insecticide which is applied to a crop in South Africa must be registered (Act 36 of 1947) for use on that crop and pest. Few insecticides are registered for use on proteas in South Africa (Vermeulen *et al.*, 1992). Only one product (dimethoate EC), is

registered for borer control on proteas. It is recognized to be an environmentally "hard" product (VOPI 1994), and using frequent application is likely to destroy parasites of borers.

3. Case study: Carnation worm on *Leucadendron* cv. Silvan Red

Carnation worm (*Epichoristodes acerbella*, Lepidoptera: Tortricidae) damage has been reported on *Leucadendron* cv. Silvan Red in the Stellenbosch area, South Africa. The larvae of this insect infest the young shoots of the plant, and later bore down into the stem, causing *ca.* 30% losses in yield (Viljoen and Wright, 1991). The pesticides registered for the control of this insect on carnations had little effect (Steenkamp, pers. comm.). A subsequent investigation into the effect of these products on the carnation worm larvae was done under laboratory conditions (Wright *et al.*, 1991). This work indicated that most organophosphate insecticides tested (parathion EC, fenthion EC, dimethoate EC), were ineffective against the insects. One product (trichlorfon WP) was effective, as well as pyrethroid products (*viz.* permethrin EC, deltamethrin EC). In addition to this, a botanical product, azadirachtin (extract of neem seed, *Azadirachta indica*, Meliaceae), was tested under field conditions. It showed limited potential, with only a soil drench application giving any significant results, of a *c.* 12% reduction in insect incidence (Wright *et al.*, 1991). The use of entomopathogenic nematodes as biocontrol agents was tested *in vitro*, with encouraging results, giving *c.* 93% insect mortality (Wright *et al.*, 1991). Their commercial application remains a problem, however. The results of this investigation indicated that: (1) use of a more suitable pesticide could control the pest; (2) an alternative product (e.g. a botanical insecticide) may have potential for inclusion in an IPM programme for this pest and (3) although nematodes have potential for use as a biological control agent of the pest, more work is needed to establish whether this is economically viable.

The larvae were also parasitized by a wasp under field conditions, with *c.* 35% of larvae harbouring parasites (van der Merwe, unpublished). The use of softer, target specific pesticides could contribute to their conservation, and the role as biological control agents could be enhanced.

4. Conclusions

Although no clear IPM guidelines are available for protea production at present, a great improvement on present conditions can be made by simply making growers aware of the need and potential effectiveness of IPM. The adoption of even rudimentary IPM practices can contribute greatly to the growth of a comprehensive IPM approach in the long term. Practices such as monitoring, the use of thresholds and use of softer pesticides should all be explored by protea growers.

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PHYTOPHTHORA DIEBACK IN BANKSIAS: SCREENING FOR RESISTANCE

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Abstract

Screening methods were investigated for assessing the resistance/susceptibility of *Banksia* species to *Phytophthora* infection. *P. cinnamomi* Rands was isolated most frequently from soil under infected *Banksia* plants in South Australia. Two isolates of *P. citricola* Sawada were also identified. Preliminary results indicated the response of excised shoot material to be quite variable and dependent on seasonal variations. The excised root assay showed potential as a routine screening assay.

Micropropagation of *B. coccinea* has been successful and material will be included in an *in vitro* inoculation assay. Research continues into the handling of *in vitro* propagated plantlets.

1. Introduction

Many Australian native species, including banksias (*Banksia* spp) have considerable potential in the nursery and cut flower industries, both in Australia and overseas (Fuss and Sedgley, 1991). There is, however, a need for continued research into development of improved varieties, with respect to size, shape, flower colour or resistance to disease. Dieback caused by certain *Phytophthora* species has hindered successful cultivation of banksias in recent years and *P. cinnamomi* poses a major threat to industry viability.

Certain horticulturally exploited species, including *B. hookeriana* Meisn., *B. coccinea* R. Br. and *B. menziesii* R.Br., were shown by McCredie *et al.* (1985) to have varying susceptibility to the fungus. Cho (1981) and McCredie *et al.* (1985) found east coast species with high levels of tolerance to *Phytophthora* infection.

Methods need to be developed to enable quick assessment of resistance or susceptibility between and within species for the selection of resistant or tolerant genotypes.

2. Materials and methods

2.1. Isolate identification

Phytophthora spp. were isolated from soil using the pear baiting technique of McIntosh (1964) and Davison and Bumbieris (1973). Pure cultures were established on cornmeal agar (CMA), identified using the 'Revised Tabular Key to the species of *Phytophthora*' (Stamps *et al.*, 1990) and identity confirmed by Dr G. Hall at the International Mycological Institute, UK.

2.2. Excised shoot assay (ESA)

Banksia shoot material was defoliated and cut into 5 – 6 cm pieces. These were surface sterilised in domestic bleach (0.8% available chlorine) and rinsed three times in sterile distilled water. Shoots were placed in tubs of CMA precolonised with *Phytophthora* for 2 weeks. Tubs were incubated in the dark at 25 °C and lesion length was measured after 10 days. Results were analysed using analysis of variance (ANOVA). Two isolates of *P. cinnamomi* (K5, K27) and one of *P. citricola* (K29) were tested.

2.3. Excised root assay (ERA)

A method used by Tippett *et al.* (1985) and Shearer *et al.* (1987) was modified for use in the experiment. Roots were excised from 9 month old *Banksia* plants, surface sterilised in domestic bleach (1% available chlorine), and rinsed three times in sterile distilled water. The cut end of each root was inoculated with a 5 mm plug of mycelium taken from the leading edge of a culture growing on CMA. Isolates of *P. cinnamomi* (K5) and *P. citricola* (K29) were tested against 15 *Banksia* species. Inoculated roots were placed on sterile, moistened vermiculite in a petri dish and incubated at 25 °C. Roots were sampled at 4, 8 and 12 days and cut into 5 mm sections. These were placed sequentially onto CMA supplemented with pimaricin, rifampicin and claforan. Length of root colonised was assessed and an ANOVA was performed.

2.4. Micropropagation of *Banksia* spp.

Micropropagation protocols are being developed for *Banksia* spp. These will be published in full at a later date.

3. Results

3.1. Isolate identification

Phytophthora cinnamomi was isolated most frequently from soil under dying *Banksia* plants in South Australia. Of the 32 isolates, only one other species was identified, *P. citricola*, which was found only twice.

3.2. Excised shoot assay

Preliminary results obtained after only the first year of experimentation showed a significant interaction between species and isolate for all species tested ($P < 0.001$, 9 d.f.).

All species showed a lower tolerance to *P. cinnamomi* than to *P. citricola*. Valuable cut flower species such as *B. hookeriana* and *B. baxteri* were highly susceptible to *Phytophthora* infection while the east coast species *B. spinulosa* var. collina appeared to have a high tolerance level. In years two and three, however, there was little lesion development and no differences were detected between species.

3.3. Excised root assay

The length of colonisation of roots of 15 *Banksia* spp. assessed after 4, 8 and 12 days indicated differences in susceptibility between the species. There were trends visible with regard to colonisation over the 12 day period. On day 8, means ranged from 17 mm for *B. spinulosa* var. collina up to 90 mm for *B. solandri* with *P. cinnamomi*. Mean length of colonisation was generally lower for all species inoculated with *P. citricola*. Lesion development for *B. spinulosa* var. collina and *B. serrata* was significantly lower ($P < 0.001$, 2 d.f.) than for other species when inoculated with both *Phytophthora* isolates. Significant differences were seen on day 8 between the isolates for several species, for example, with *B. sceptrum*, *P. cinnamomi* (K5) was significantly more aggressive than *P. citricola* (K29)($P < 0.01$, 1 d.f.). Differences in susceptibility to both isolates were also seen between several *Banksia* species. Since only 2 – 3 species were inoculated in any one experiment, analysis can be performed only within these subsets. For example, a significant difference was seen between *B. sceptrum* and *B. ericifolia* ($P < 0.05$, 1 d.f.) over the 12 day period.

3.4. Micropropagation of *Banksia* spp.

The response of *Banksia* material *in vitro* has been varied. Survival rates have been encouraging with selected species and genotypes (*B. coccinea, B. hookeriana* and B. spinulosa var. *collina*. Multiplication, elongation and rooting of *B. coccinea* are in progress.

4. Discussion

The isolation of *P. cinnamomi* from the majority of hosts examined indicates that this species was most active in causing disease of *Banksia* species at the time of year sampled.

The excised shoot assay is a quick method, but appeared to depend on the type of material used and the year selected. Several species including *B. hookeriana, B. baxteri* and *B. prionotes* have also been shown by other researchers, using whole plant inoculations, to be highly susceptible (McCredie *et al.*, 1985; Cho, 1983; Dixon *et al.*, 1984). However, *B. brownii, B. ashbyi*, and *B. menziesii* are species previously shown to have moderate to high susceptibility to *Phytophthora* infection whereas small lesions only were observed here in years 2 and 3. This method is, therefore, not considered to be a reliable screening technique.

The results obtained using the excised root assay were similar to those found with the ESA in year 1. Several east coast species, including *B. spinulosa* var. collina, showed tolerance to infection while the valuable cut flower species, *B. hookeriana* and *B. menziesii*, were highly susceptible. Infection in most species correlated well with that reported by McCredie *et al.* (1985) and Dixon *et al.* (1984). Assessments made at day 8 gave the best correlation with the ESA and results of other researchers.

Screening for resistance to *Phytophthora* infection may be possible *in vitro*. Resistant and susceptible material is being multiplied for *in vitro* inoculation experiments using various *Banksia* species and *Phytophthora* isolates. This approach

would allow results to be obtained routinely in the shortest possible time. Research is also aimed at *in vitro* methods for clonal propagation of improved *Banksia* genotypes.

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CULTIVAR DEVELOPMENT OF ORNAMENTAL MEMBERS OF THE PROTEACEAE

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Abstract

Ornamental members of the family Proteaceae are now well established in the cut flower and amenity industries of many countries around the world. As with all ornamentals, however, it is imperative that we continue the development of new lines and products to keep pace with the ever-changing market. The most important features of any new cultivar are novelty, quality, yield and reliability. Research is an essential tool in understanding the biology of the plant, and in using this information in product improvement. These problems are discussed in terms of cultivar registration and evaluation, and illustrated using *Banksia* breeding as an example.

The *Banksia* selection program is currently focused on the cut flower species *B. coccinea, B. menziesii, B. hookeriana* and *B. prionotes*, and two superior cultivars have so far been identified. 'Waite Orange' is a high yielding orange banksia with a production time intermediate between that of *B. prionotes* and *B. hookeriana*. Using a combination of *B. prionotes*, 'Waite Orange' and *B. hookeriana*, orange banksias can be produced almost year round. 'Waite Crimson' is a high yielding, mid-season *B. coccinea* with dark red blooms. Vegetative propagation techniques, via rooted cuttings, have been developed for both cultivars. The program is also aimed at interspecific hybridisation within the genus, to combine characters of interest across species barriers and so produce novel cultivars. Research conducted on the structure and morphology of the pollen presenter and pollen grains across the genus has shown that there is variability in the structure of both, but particularly in the pollen presenter. There are four major types of pollen presenter, elongated and cylindrical, short and cylindrical, short and ovoid, and papillose. The location of the stigmatic groove also varies between species, with most terminal and longitudinal, but some subterminal and/or transverse in orientation. It is important to specifically target the stigmatic croove in interspecific pollinations, as the pollen-receptive cells lie within the groove.

1. Introduction

Proteaceous blooms are now well-established as an important component of the international cut flower trade. The main genera are *Protea, Leucospermum, Leucadendron* and *Serruria* from South Africa, and *Banksia* and *Telopea* from Australia. All genera are relatively new in the industry, and quality criteria and standards are still evolving. The main advantage of proteaceous blooms is their conspicuous and showy nature, which renders them ideally suited as standard blooms which form the focus or centrepiece of large floral arrangements. This prominence also means that the quality of the bloom is paramount, and achieving this quality is one of the main challenges facing plant breeders.

It is important that plant breeders develop strict selection criteria for their crop, in collaboration with the relevant industry. This provides clear goals toward which the

breeder can aim. It is imperative, however, that these goals are far-sighted. The cut flower industry is subject to changes in consumer preferences, and plant breeding is by nature a long-term undertaking. Sensitivity to and appreciation of potential future trends in the industry must be taken into account in the development of the selection criteria. Novelty is an important factor in success, and the breeder must exploit available germplasm to maintain the flow of new and interesting cultivars. Some of the problems and challenges facing breeders of proteaceous crops will be illustrated using the Australian genus *Banksia* as an example.

2. Selection criteria

The establishment of clear, strict selection criteria is one of the most important stages of the breeding program. For most proteaceous crops the important selection criteria are as follows:

1. Yield
2. Quality
3. *Phytophthora* tolerance

3.1. Yield

The number of blooms per bush, and hence per unit area, is one of the major factors contributing to the economic viability of a proteaceous planting. Properties established from seed-grown plants typically show enormous variability in all characters, including yield (Sedgley *et al.*, 1991). As land becomes more expensive, through conflicting land-use demands such as housing, and in some countries through land degradation, it is increasingly important to maximise returns per unit area. Once yield gains have been achieved via breeding and selection, it is essential that vegetative propagation methods are developed to capture the gains and ensure yield realisation throughout the planting. Also important is research into crop management, including irrigation, nutrition, pruning and pest and disease control aimed at yield optimisation (Sedgley and Fuss, 1992; Luke, 1991; Seaton and Woods, 1991; Cresswell, 1991).

2.2. Quality

The industry must develop strict quality standards if it is to maintain and expand its share of the international market in competition with established crops such as rose, chrysanthemum and carnation. This is underway within the industry over the range of proteaceous crops, and for certain species of *Banksia* as part of the Australian *Banksia* selection and breeding program (Fuss and Sedgley, 1991a; Sedgley, 1991). The most important criteria are as follows:

1. Long straight stem for ease and flexibility of use by the florist
2. Terminal bloom with no interfering foliage to obscure the appearance of the inflorescence
3. Even bloom with minimal floret abortion
4. Attractive floret colour

5. Complementary foliage with attractive colour and leaf shape
6. No pest or disease damage

In the case of *Banksia coccinea* and *B. menziesii*, these criteria have been quantified (Fuss and Sedgley, 1991a). Export quality blooms must have a stem length, including the inflorescence, of at least 60 cm for *B. coccinea* and 50 cm for *B. menziesii*, with an inflorescence length of at least 6 cm and 10 cm respectively. The offset angle of the inflorescence must be no more than 15° from the vertical, and there must be less than 5% abnormal floret development with no leaf damage.

The requirement for a terminal inflorescences dictates the species of *Banksia* which are cultivated for cut flowers. Nine of the 75 species of *Banksia* provide the bulk of the crop, all of which are native to western Australia. These are *Banksia coccinea, B. menziesii, B. baxteri, B. hookeriana, B. prionotes, B. burdettii, B. victoriae, B. ashbyi* and *B. speciosa*. Many of the lateral-blooming species have attractive inflorescences, but their value is reduced because of interfering foliage and bent peduncles. While evenness of bloom appears to have a genetic basis, there may also be an environmental component. More abnormal floret development was observed in *B. coccinea* grown in cool sites (Fuss and Sedgley, 1991a), and temperature was also suggested to cause inflorescence abortion in *B. menziesii* (Fuss *et al.*, 1992). Thus, site selection is an important management decision in *Banksia* production. Red is the most popular colour for cut flowers, and this is reflected by the dominance in the banksia market of *B. coccinea*, the scarlet banksia. Variability for inflorescence colour provides the breeder with the opportunity to select for this character, with variation in *B. coccinea* from pink to burgundy and from orange to scarlet, and in *B. menziesii* from yellow to wine-red, with pink the most common colour (Sedgley *et al.*, 1991). The importance of leaf shape and size is demonstrated in the orange banksias, with the fine leaves of *B. hookeriana* preferable to the large coarser leaves of *B. prionotes*. Selection against pest and disease damage to the cut bloom is very difficult in the absence of detailed knowledge regarding the problem organisms and their life cycles, but this will assume greater importance as the industry develops and as more information becomes available. This is particularly important for the export market, in view of restrictions in some countries on the import of infested material (Coetzee and Wright, 1991).

2.3. *Phytophthora* tolerance

The root rot fungus *Phytophthora cinnamomi* is one of the major limitations to the cultivation of proteaceous plants for cut flower production. Its prevalence can be controlled by management (Turnbull, 1991; Sivasithamparam, 1991), but the devastating nature of the fungus is such that tolerance to the disease must be included in breeding programs as an important selection criterion. Our research has shown that *P. citricola* is also an important pathogen of *Banksia* species (Tynan *et al.*, 1993), and a number of other *Phytophthora* species has been isolated in western Australia (Sivasithamparam, 1985). Research is currently underway to develop routine screening techniques to test for tolerance to the fungus, involving both excised shoot and root assays (Tynan *et al.*, 1995). This approach is showing promise, and the methods should be readily adaptable to other members of the Proteaceae, and even to other families.

3. Cultivar registration and commercialisation

Many countries now have legislation to protect the rights of the plant breeder, and to allow the collection of a royalty on improved material. In Australia, Plant Variety Rights legislation was introduced in 1987, and the royalty is collected on each plant sold. Many breeders are not commercial propagators, so the owner of a new variety may sub-licence a nursery to multiply and market the plants. As the sublicencing company is making a financial commitment to the variety, it is often the case that overseas rights are delayed, to allow the local market a production edge. This has implications with regard to testing of new varieties over a range of environments.

Positive identification of the plant genotype is an important aspect of cultivar protection and breeder rights. Most new cultivars are identified by their floral characteristics, and this limits identification to the flowering period of the plant. In addition, there is environmental variation in many plant characteristics which may confound positive identification. To overcome these problems, breeders are increasingly turning to biochemical identification, using characters which are not subject to environmental or tissue-specific variation. The most commonly used methods are those which detect variation in enzymes, proteins or nucleic acids by electrophoresis of plant extracts (Ashari *et al.*, 1989). A characteristic fingerprint can be developed for each genotype, which can be verified at any time of year. These techniques can also be used to investigate relationships between breeding lines and parentage of cultivars.

4. Selection and breeding methods

The South African and American selection and breeding programs for Proteaceae have resulted in many excellent cultivars which form the basis of the international industry (Parvin, 1981; Brits *et al.*, 1983). These programs have concentrated on the African genera, and development of Australian members of the Proteaceae for cut flower production is very recent (Sedgley *et al.*, 1991). The Australian *Banksia* selection program is currently focused on the species *B. coccinea*, *B. menziesii*, *B. hookeriana* and *B. prionotes*, and two superior cultivars have so far been identified. 'Waite Orange' is a high yielding orange banksia with a production time intermediate between that of *B. prionotes* and *B. hookeriana* (Sedgley, 1991). Using a combination of *B. prionotes*, 'Waite Orange' and *B. hookeriana*, orange banksias can be produced almost year round. 'Waite Crimson' is a high yielding, mid-season *B. coccinea* with dark red blooms. Vegetative propagation techniques, via rooted cuttings, have been developed for both cultivars. Clonal propagation is essential for reliable production of top quality blooms.

The South African and American programs have both employed interspecific hybridisation as the basis of cultivar development (Parvin, 1981; Brits, 1985a, 1985b; Brits and Van Niekerk, 1985). The Australian program is aimed at interspecific hybridisation within the *Banksia* genus, to combine characters of interest across species barriers and so produce novel cultivars. Hybridisation methods have been developed, based on research into the breeding system of the genus (Fuss and Sedgley, 1991b). Research conducted on the structure and morphology of the pollen presenter and pollen grains across the genus has shown that there is variability in the structure of both, but particularly in the pollen presenter (Sedgley *et al.*, 1993). There are four major types of pollen presenter, elongated and cylindrical, short and cylindrical, short and ovoid.

and papillose. The location of the stigmatic groove also varies between species, with most terminal and longitudinal, but some subterminal and/or transverse in orientation. It is important to specifically target the stigmatic groove in interspecific pollinations, as the pollen-receptive cells lie within the groove.

5. The future

The continued international interest in Proteaceous cut flower crops provides the challenge for plant breeders to supply new cultivars. This goal will be facilitated by a number of developments which require action over the next decade. One of the most important factors is international co-operation in all aspects of cultivar development.

5.1. Common international quality standards

Efforts within individual producer countries, to develop quality standards based on the requirements of consumer countries, should be coordinated internationally. These standards can then be used to set the selection criteria for individual breeding programs. This approach could also be extended to the development of an international marketing strategy. Northern and southern hemisphere countries, with their different periods of peak harvest, should be seen as complementary rather than competitive producers. This approach would reduce commercial limitations in exchange of breeding lines and availability of new cultivars.

5.2. Cultivar identification

This will become increasingly important as the range of cultivars increases and material is distributed around the world. The new methods of genotype identification using DNA technology are currently being used successfully on *Banksia* species, and are thus available to breeders of Proteaceous crops.

5.3. Breeding systems research

The genus *Leucospermum* has so far proved the most flexible in terms of cultivar production via interspecific hybridisation (Brits and Van den Berg, 1990). Many of the other commercial genera are more difficult to manipulate, but are equally spectacular in terms of the cut flower market. Detailed research into the breeding systems of Proteaceous genera will assist in the development of hybridisation methods to produce a wider range of cultivars.

5.4. Genetic engineering

This exciting area of research provides the promise of transfer of useful genes across taxonomic barriers. While research into gene isolation and control is quite advanced in a number of organisms, there is a major bottleneck with many plants as regeneration *in vitro* is generally required for transformation. Thus, an important goal of Proteaceous research should be *in vitro* regeration of whole plants. When this has been achieved, then the way will be open for genetic manipulation and transformation

of Proteaceous genera. Flower colour is a character which could potentially be altered, and in the future manipulation of more complex features such as flowering time or growth rate might also be possible.

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'MARKETABLE PRODUCT' APPROACH TO BREEDING PROTEACEAE IN SOUTH AFRICA

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Abstract

Proteaceae breeding has a history of about 25 years in South Africa. Within this time span, the need to apply certain basic principles of breeding have become evident. It is possible to divide the genera *Protea, Leucospermum* and *Leucadendron*, into well defined marketable products. Within each genus each of the marketable products defines a primary breeding aim. The likelihood of success of developing each of the product lines within the genera is dependent on the ease of hybridization within and between species. Improvement of the products within genera can be divided according to the probability of improvement by intraspecific hybridization. Within product lines, where improvement is limited if using only intraspecific hybridization, interspecific hybridization will have to be used. The latter can result in two types of progeny, namely, improved product line, or unique product unlike any currently on the market. Ultimately the rate of progress within each product line will depend on the ability to overcome inherent problems of low seed set and cross incompatibility between species.

1. Introduction

One of the most interesting and diverse plant families found in southern Africa, from a botanical and commercial point of view, is the Proteaceae family. Many of the species within this family have developed into valuable commercial cut flowers, not only in South Africa, but also in other climatically similar regions of the world.

In the early stages of developing the industry, protea flowers were harvested from natural plant stands. However, other countries with similar climatic conditions developed cultivated proteas of a higher picking quality. The lower quality veld picked flowers are less desirable on the international market and to overcome this resistance to South African produced proteas, a need arose to develop protea farming techniques in South Africa. Of initial importance was the development of horticultural practices to enable efficient cultivation. However, as with many 'wild' plants, proteas are not entirely suitable for cultivation and in many cases the only method of improvement lies in directed breeding efforts.

This paper presents an overview of the current approach to breeding *Protea, Leucospermum* and *Leucadendron* at the Fynbos Research Unit.

2. Materials and methods

Breeding efforts at the Fynbos Research Unit concentrate on the genera *Protea, Leucospermum* (pincushions) and *Leucadendron* (cone bushes), with some selection and evaluation also being done in *Serruria*.

Conservation of the germplasm required to breed new types is done by cultivation in

plantations at Elsenburg Agricultural Development Institute near Stellenbosch in the South Western Cape. This collection of plants includes commercially grown cultivars, variants of commercially grown species and variants of species with no commercial value but which possess desirable genetic characteristics such as tolerance of *Phytophthora* root rot (Brits, 1990). The plants in the collection are evaluated for their appearance, yield characteristics and commercial worth and are used as parents in the production of hybrid seed. This hybrid seed may be from crossing variants of the same species (intraspecific), or from crosses between species (interspecific). The crosses are planned with the objective of selecting progeny which fall into one of the marketable product categories defined in table 1.

The process through which all selections or new seedlings must pass is as follows: selected variants and seedling progeny pass through three stages of evaluation before a decision is taken to release a new cultivar. In PHASE I the floral characteristics of the plant and time of flowering are described and the plant is visually judged for marketability. Cuttings are taken from suitable plants. These, when planted out, are evaluated as PHASE II plants. In this phase evaluation is for time and duration of flowering, morphology, vigour and yield during three consecutive years from the first year of flowering. If a specific line passes the PHASE II evaluation the final stage is ELITE evaluation of candidate cultivars. This involves evaluation for yield, travel-ability of the product, vase life and market acceptance. Optimally the ELITE evaluation should be an "on-farm" operation, whereby protea farmers plant small evaluation production blocks under contract for the Fynbos Research Unit. The information required from these blocks is the method of cultivation, time of marketing, marketable harvest, travel-ability and price obtained per stem when marketed. Simultaneously the evaluation including vase life is done at Fynbos Research Unit. In this way the protea farmers, the intended end users of cultivars, can contribute directly to the quality and suitability of the cultivars released by the Fynbos Research Unit. Using the information supplied by farmers, a more complete data-base can thus be built up about the candidate cultivar before release, making the eventual choice of which cultivars to grow where much easier.

3. Discussion

The Proteaceae are open pollinated plants in which seed populations produce a diversity of types, differing in characteristics such as vigour, flower colour, growth habit and yield (Brits, 1984). Selection of single plants from within a seed population and clonal multiplication by cuttings of the single plant can give rise to new cultivars. In some cases farmers are able to complete this process themselves. Such cultivars can be of benefit to individual farmers, but it takes many years before they may be used by other farmers. Thus the benefit for the industry as a whole is limited. Species selections from intraspecific seedling populations developed at Fynbos Research Unit are available on request to all farmers.

Characteristics, such as vigour, travel-ability and vase life, and extension of flowering period can only be improved by hybridisation. In orchards where different species and ecotypes are grown together it is possible to select novel hybrids from seed harvested, but theoretically much greater progress can be made by deliberately crossing plants with desirable characteristics. In the Proteaceae family there are constraints of

low seed set (Horn, 1962) and lack of seed set after interspecific hybridization (Brits and Van den Berg, 1990) which hamper progress. These constraints are being studied at the Fynbos Research Unit.

3.1. Genus *Protea*

In product lines such as *P. cynaroides*, which has many ecotypes which collectively flower throughout the year (Brits *et al.*, 1983), the most progress will be made by making intraspecific crosses between the different ecotypes. The aim is to have cultivars available in each colour and size range that flower successively throughout the year. The product types *P. eximia*, *P. compacta* and *P. repens*, however, will require interspecific hybridisation to extend the flowering period into the summer months and to help overcome problems of post-harvest leaf blackening. This can result in two types of progeny, namely, improved product line, or unique product unlike any currently on the market. Product types *P. magnifica* and *P. neriifolia* will be able to be used to upgrade each other, which due to their close relatedness will not present too many difficulties. Small types of protea can only be developed by interspecific hybridisation and this is an area with great potential.

3.2. Genus *Leucospermum*

In *Leucospermum* the most desirable flower head type is that of *L. cordifolium* or *L. cordifolium* hybrids. The primary aim is to develop series of cultivars in the colour ranges given in table 1 that flower successively from as early in the season to as late as possible. Predominant use of interspecific hybrids in the breeding programme will increase the adaptability, vigour, yield and root disease tolerance of the cultivars.

3.3. Genus *Leucadendron*

In *Leucadendron* the genetic variation available is vast and as yet largely untapped. Hybridization experiments (Brits and Van den Berg, 1990) have shown that there are fewer limitations to interspecific hybridisation within *Leucadendron* than in other genera. The goals set out in table 1 can be relatively rapidly achieved. Again the aim is to have a long marketing period for each product, either due to the long period over which a cultivar may be harvested, or by using cultivars with different flowering or colour changing times, but similarity in other respects.

The techniques to successfully hybridize species at will are not yet available and may never become reality. Steps to discover why it is difficult to produce hybrids artificially while chance hybrids seem to crop up constantly, are under way. The breeding of protea presents great challenges and many economic benefits if the end results are favourable. Ultimately the rate of progress within each product line will depend on the ability to overcome inherent problems of low seed set and cross incompatibility between species.

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Table 1. Marketable products within the *Protea*, *Leucospermum* and *Leucadendron* genera.

Genus	Type	Colour line	Size/shape
Protea	*repens* sugarbush	red checker white	each of these colour lines in large, medium and small
	cynaroides king protea	pink/red red white	
	magnifica barbigera	large medium small	
	neriifolia	red white	
	compacta	red white	
	eximia small types		
Leucospermum	cordifolium	red yellow orange pink	each of these colour lines in large, medium and small
Leucadendron	foliage	red	chrysanth rose-bud tulip
		green	chrysanth rose-bud tulip
		yellow	chrysanth rose-bud tulip
	single stem	red	chrysanth rose-bud tulip
		green	chrysanth rose-bud tulip
		yellow	chrysanth rose-bud tulip

INTERNATIONAL PROTEA CULTIVAR REGISTRATION: PROGRESS REPORT

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Abstract

Being the International Registration Authority for proteas of South African origin, the Department of Agriculture of South Africa maintains the International Protea Register (IPR) and checklist of validly published protea cultivar names. Following a slack period of several years, international cultivar registration has resumed, resulting in an increase in the number of registered cultivars to 47 at present. A draft checklist, based on the original 'Sample List' of G. Brits, was compiled with a 40% increase in cultivar names. However, the lack of information is still a problem, as well as poor international co-operation. The knowledge and co-operation of the international protea industry is, for instance, needed to verify the information in the checklist for correctness and to rectify the imbalance between the number of cultivar names in the industry that has not been registered and the number of names registered in the IPR. If breeders need to protect their cultivars from exploitation, they should apply for Plant Breeders' Rights (PBR) in each of the protea growing countries where they want protection. With a breeder's right granted, the owners enjoys protection of the cultivar and receives remuneration (royalty payment) on all plant material sold of the particular cultivar.

1. Introduction

The topic of international protea registration has been discussed and presented at various occasions since the early 1980's. However, it did not result in the international co-operation and registration of protea cultivars as it ideally should have. Brits (1988) promoted the system by writing articles and presenting lectures at conferences. Apart from numerous other publications, Protea News 7 (1988) was devoted entirely to the registration of protea cultivar names.. The two systems of registration, namely the International Protea Register (IPR) and Plant Breeders' Rights (PBR), have been discussed in length. Despite these efforts, it is still mainly cultivar names from South Africa that appear in the IPR. The aim of the IPR is to promote uniformity, accuracy and stability in the naming of cultivars and to avoid confusion and duplication of cultivar names.

The importance of the registration of cultivar names for the international protea industry cannot be stressed strongly enough. If an organised and successful protea industry is the goal of protea growers and breeders, they should no longer hesitate to register their cultivars. A successful international register which could be taken as an example, is the International Rhododendron Register. The International Registration Authority (IRA) for Rhododendron started with their register in 1958 and has already published the twenty-ninth supplement to that register in 1989.

2. International Protea Register

The International Protea Register (IPR) is supposed to contain the registered names of cultivars commercially available in the protea growing countries. Unfortunately, only 47 cultivar names, of which only 7 are not from South Africa, are currently registered. The IPR has a number of advantages, some already mentioned, as well as to avoid duplication of names and to ensure nomenclatural stability. Other advantages include protection of the cultivar name and free promotion. A registered name may not be used for another cultivar. In case of duplication of a cultivar name which has been registered in the IPR, the registered name is valid and another name has to be selected for the duplicating cultivar. All protea breeders/growers are urged to ensure that their cultivar names are preferably registered *before* being released or catalogued. The earlier a cultivar name is registered, the less chance there is to lose the name because of duplication. However, it is not too late to register the names of unregistered cultivars already in commerce. The aim is to have all cultivars that are commercially available, registered in the IPR.

The first publication of the IPR appeared in the South African Plant Variety Journal (SAPVJ) in 1986. It was decided to use the SAPVJ to publish all matters concerning the IPR due to the lack of a publication devoted exclusively to the IPR. Unfortunately this resulted in the relevant information not reaching the protea growers/breeders. The publication of applications and decisions on applications will be continued in the quarterly SAPVJ. However, the complete IPR listing all the registered cultivar names with information such as the parentage, breeder/originator, date of origin, introducer, date introduced and registered as well as a short description of the flower will in future be an individual publication distributed to interested persons. Those interested are requested to forward their names and addresses to the IRA to be put onto the mailing list for the IPR.

In order to have a cultivar registered in the IPR, certain conditions have to be met. A fully completed application form including a colour slide/photograph of the flower and of the plant as well as the application fee should be sent to the IRA. The other condition is that the cultivar must be commercially distinct from all the cultivars already registered in the IPR. The descriptions and photographs of the candidate cultivars presented to the IRA in the application forms are compared to that of the comparable cultivars registered in the IPR. If no differences can be found between the candidate cultivar and one or more of the cultivars in the IPR, the applicant will be advised to forward more detailed information to the IRA. If the applicant fails to forward the required information or cannot supply significant evidence of differences, the application will be rejected. When an application is successful and accepted for registration, the applicant will be informed accordingly. In future a certificate will be issued for each cultivar name registered. The IRA is still working on this matter and hope to implement it soon.

Application forms as well as any information required, can be obtained from the office of the IRA. The following addresses should be used:

- International Registration Authority: Proteas, attn. Ms Joan Sadie, Directorate of Plant and Quality Control, Private Bag X5015, Stellenbosch 7599, South Africa; or alternatively,

- International Registration Authority: Proteas, attention Mr Martin Joubert, Directorate of Plant and Quality Control, Private Bag X258, Pretoria 0001, South Africa.

Due to the enormous task of the IRA to maintain the international register as well as the international checklist, regional co-ordinators (RCs) were appointed to assist the IRA and provide the necessary contact between the IRA and the breeders/growers. Application forms and information regarding protea registration should also be obtainable from the Regional Co-ordinators. They are:

- Mr David Matthews, P O Box 18, Monbulk, Victoria 3793, Australia
- Dr George Mason, Sutton Road RD4, New Plymouth, New Zealand
- Mr Dennis Perry, Perry's Panorama, P O Box 540, Somis, California 93066, USA
- Dr Jaacov Ben-Jaacov, A.R.O. The Volcani Research Center, P O Box 6, Bet Dagan 50250, Israel
- Mr Trevor Hedges, Department of Crop Science, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe.

3. International checklist of protea cultivar names

In accordance with the instructions of the International Society for Horticultural Science (ISHS), the IRA has the duty to compile and maintain an international checklist of validly published protea cultivar names. This way record can be kept of the valid cultivar names which have not been registered in the IPR as well as of those that have been discontinued to avoid situations where valid names are duplicated. The International Code for Nomenclature of Cultivated Plants (in short: Horticultural Code) states clearly that a valid name should not be used for another cultivar, even though this cultivar may not be in circulation anymore. To be considered validly published, a cultivar name must have been published in books, journals, brochures or other printed matter available or distributed to the public and in which the publishing date is clearly noted. Publication of a cultivar name must include a description of the cultivar or reference to a previous description as a cultivar; the cultivar has to be compared to and distinguished from related cultivars; the parentage, history and name of the breeder and/or introducer have to be mentioned; and where possible, the description should be elucidated with an illustration. It is important to remember that publication of a cultivar name is not valid if it is published against the wish of the originator and if the cultivar itself does not or did not exist.

The draft checklist is based on the 'Sample List' compiled by Brits (1988). The number of cultivar names in the checklist has been increased to ± 230 with the help of the valuable contributions of a few respondents and by processing a number of publications at our disposal. Most of the information was retrieved from the book by Matthews and Carter (1983), while catalogues, journals and posters were also valuable. Information concerning the parentage, date of selection, country of origin, reference to the source of publication, the breeder/introducer, the status (e.g. cut flower) and remarks which contain additonal information on the cultivars are included in the checklist.

Despite the number of names and other information already added to the checklist,

additional information such as the country and date of origin, breeder/introducer and especially the parentage and source of publication are still required (table 1). With all this information included in the checklist, a number of questions and problems could be resolved.

The occurrence of duplicate names is a serious problem and should be attended to without delay (table 2). The decision on whether a cultivar name is valid and whether it has priority, should be made according to the Horticultural Code. A registered name has the highest priority, whether either PBR or IPR is applicable. Valid publication is next on the priority ranking followed by the dates of publication.

Due to registration in the IPR, four duplicated names enjoy priority. They are *Protea* 'Cardinal', *Protea* 'Red Baron', *Leucospermum* 'Mars' and *Leucospermum* 'Yellow Bird' (table 2). The validity of the other cultivars with duplicated names will be determined by valid publication and the dates of publication. The owners of these cultivars should attend to this matter as soon as possible. Duplicate names should either be changed or the cultivars discontinued, as happened with cultivars 'Fantasy' and 'Riverlea' (table 3). After publication of the 'Sample List' (Brits, 1988), Mr Jack Harré took action by changing the name of *Protea* 'Fantasy' to 'Pink Fantasy' and discontinued production of *Leucadendron laureolum* 'Riverlea' (Harré, 1989).

Another cause of concern is the incomplete information on the parentage of cultivars originating from hybridization (table 4). In some publications parentage is indicated by the word "hybrid" or only one parent is mentioned, while other publications mention both parents, e.g. in the IPA Journal (1991) the parentage for 'Meteor' is indicated as *Leucospermum lineare* x *Leucospermum vestitum* while the nursery catalogue of Duncan & Davies (1978) only mentions *L. vestitum*. Could it be that later evidence was found to identify the hybrid combination or is it duplication? In the case of 'Cloudbank Jenny' (*Leucadendron discolor* x *L. gandogeri*) another question arises, because the parentage of 'Cloudbank Ginny', also in the USA, is indicated as *Leucadendron* hybrid. Is it coincidence that the parentage of one cultivar is known while the other is not or is it a matter of ignorance/misunderstanding concerning the pronunciation of the name.

The IRA cannot make assumptions nor should decide which is the correct information. The help of each and every breeder and grower is needed to work through the checklist, verify all the information and forward the corrections and amendments to the IRA at the earliest possible date. The IRA relies on the co-operation of the international protea industry.

4. International co-operation

One of the most important requirements of the registration system to be successful, is the positive and reliable co-operation of all participants in the industry. During an attempt by the IRA to gather information for the checklist, nineteen protea growers/breeders and RCs in the protea growing countries were approached, resulting in a total of five responses received from Australia, California, Hawaii and New Zealand. Two persons not only presented information for the checklist, but also applications for the IPR. However, the lack of positive co-operation from most people in the protea industry is a serious problem. From time to time letters appeared in the IPA Journal expressing concern about the growing disorder, especially in the naming of cultivars. Despite these and other calls upon the growers/breeders to register their

cultivars, there are still a large number of cultivars commercially available which have not been registered in the IPR. It is alarming considering that ± 230 names appear in the international checklist while only 47 cultivar names are registered in the IPR! The co-operation of the breeders/introducers as well as the RCs is essential. The RCs should constantly be aware of the movement of cultivars and publications of new cultivars in their regions and they are expected to inform the IRA on a regular basis about all protea cultivar news.

5. Plant Breeders' Rights (PBR)

The system of PBR had its origin in the International Convention for the Protection of New Varieties of Plants, as revised since its signature in Paris on 2 December 1961. The Union for the Protection of New Varieties of Plants (UPOV) was subsequently founded and is an intergovernmental organization based on the International Convention. The headquarters of UPOV is situated in Geneva, Switzerland. The Union currently consists of 24 member States (Table 5). The protea producing countries are Australia, Israel, New Zealand, South Africa, Spain and the USA (California and Hawaii).

The objective of the Convention is the protection of new varieties of plants by means of legislation. Each member country of UPOV has national laws in conformity with the Convention. A plant variety (cultivar) is considered new if it is clearly distinguishable from any other cultivar whose existence is a matter of common knowledge at the time protection is applied for in a particular country. The cultivar itself may be known at that time, but may not have been offered for sale or marketed in the country where the applicant/breeder seeks protection. However, application cannot be made for PBR if the cultivar has been available for more than one year in the country where protection is sought and not more than six years elsewhere in the world. Other requirements are uniformity and stability. The cultivar must be sufficiently uniform in its relevant characteristics and these characteristics must remain unchanged after repeated propagation to prove its stability.

6. Why legal protection?

Breeding of new cultivars requires substantial amounts of skill, labour, time and money. Breeders usually expect reward for the expensive and time-consuming work they have undertaken. However, by simply selling propagative material they cannot secure profitable reward since other people may just as easily propagate the new material themselves for further sale. In order to protect breeders against such exploitation, a plant breeder's right or plant variety right is granted, permitting the breeder alone to produce and/or sell propagative material for a limited period of time. The breeder can authorize production or sale by other agents by means of issuing a licence to such a person. This ensures that the breeder has control over the propagating material of his/her cultivar. He is able to collect royalties on all propagating material whether sold or produced by a grower. The breeder is also protected against competitors who might produce propagating material of inferior quality. PBR is granted to encourage plant breeding and, most important, to do justice to the breeder by giving him the opportunity to receive a well deserved remuneration for his labour and investment.

7. Protection at national and international level

Two types of protection for plant breeders exist, namely patents and plant variety rights/plant breeders' rights. The legal systems differ from country to country, but basically they have certain factors in common. A plant breeder's right is granted for a limited period of time. According to the revised Convention of 1991, the period is a minimum of 25 years. Rights granted under national laws are limited to the territory of that particular country. In other words, PBR are limited to and are only valid in, the country where granted. Breeders desiring protection in more than one country, have to apply for PBR in each country according to its particular law, language and fees. The international agreement on the Convention, however, enables breeders from the member countries to file applications for protection in the different countries where they can expect to receive the same treatment as in their own countries. It is important to remember that a non-resident in a particular country cannot apply for PBR in that country unless through an agent or patent attorney to act on behalf of the applicant/breeder.

8. PBR *vs.* IPR

PBR offers protection on the propagative material of the particular cultivar as well as the cultivar name attached to the material. Registration in the International Protea Register on the other hand offers protection to the cultivar name only, i.e. the Registrar has the obligation to maintain order in the international industry. PBR is only valid in the countries where applied for and granted, while registration in the IPR is done once only and is valid internationally. One could say that the IPR functions as the "international cultivar list". Due to the fact that the Department of Agriculture of South Africa is the International Registration Authority for Proteas, applications for the grant of PBR in this country should, as a matter of course, be accompanied by an application for registration in the IPR. In other countries PBR and IPR registration operate independently. However, it is advisable that while applying for PBR in a country other than South Africa, the breeder should simultaneously apply for registration in the IPR. These combined actions will help to avoid the situation where the majority of cultivars in circulation are not registered in the IPR and will bring the two registration systems into harmony.

9. Conclusion

The International Protea Register is being operated for the benefit of the international protea industry with the intention of providing a system of order. By using the IPR and the checklist, breeders/introducers could gain first-hand information on existing cultivar names and on the availability of cultivars. Breeders can benefit from the international registration of protea cultivar names in that protection of the name and free promotion is offered. Protection can be extended further to the level of plant breeders' rights where the propagating material is protected. In essence, the success of the international protea cultivar registration system depends on the international co-operation by the protea growers, breeders and Regional Co-ordinators.

Breeders are once again urged to register their cultivars, whether they are new

cultivars or those already commercially available. In the case of duplicated names, the cultivars that are registered in the IPR before others, or, if not registered, the names that have been validly published first, will enjoy priority. Another urgent appeal is made to the people in the international protea industry, to check the information in the checklist of protea cultivars and forward the correct information, where applicable, as soon as possible to the IRA. The IRA has an obligation to serve the international protea industry and is striving to provide and maintain a successful international protea cultivar registration system. However, this will not be possible without the valuable help of all concerned.

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Table 1. Number of cultivars with missing information in the different categories of the international checklist, also expressed as percentage.

Category	Number of cultivars	Percentage
Breeder/Introducer	140	63%
Country of origin	16	7%
Date of origin	191	86%
Parentage	18	8%
Source (publication)	33	15%

Table 2. Duplicate names occurring within and between countries and genera.

Cultivar name	Parentage	Country	Publication
'Bridesmaid'	*S. florida* x *S. rosea*	South Africa	SAPPEX News '92
'Bridesmaid'	*S. rosea*	New Zealand	Matthews '93
'Cardinal'	*Ld. salignum*	Australia	Australian Poster '87
'Cardinal' (IPR)	*P. eximia*	South Africa	SAPPEX News '88
'Fantasia'	*P. hybrid*	New Zealand	Riverlea '86
'Fantasia'	*P. magnifica*	New Zealand	Matthews '92
'Firefly'	*Ls. cordifolium*	—	IPA Poster '91
'Firefly'	*Ls. tottum* x *Ls. vestitum*	South Africa	Landbouweekblad '78
'Honeyglow'	*Ls. cuneiforme*	New Zealand	Duncan & Davies '78
'Honeyglow'	*P. repens*	Australia	—
'Mars' (IPR)	*Ls. cordifolium*	South Africa	SAPPEX News '81
'Mars'	*P. magnifica*	South Africa	SAPPEX News '85
'Princess'	*P. grandiceps*	Australia	IPA Poster '91
'Princess'	*P. magnifica* x *P. longifolia*	South Africa	—
'Ruby'	*Ls. cordifolium*	New Zealand	IPA Journal '86
'Ruby'	*P. neriifolia*	New Zealand	Riverlea '86
'Yellow Bird'	*Ld. salignum*	New Zealand	Duncan & Davies '78
'Yellow Bird' (IPR)	*Ls. cordifolium*	South Africa	Landbouweekblad '79

Table 3. Duplicate names which have been corrected.

Cultivar	Parentage	Country	Publication	Action
'Fantasy'	*Ls. tottum*	New Zealand	Duncan & Davies '78	
'Fantasy'	*P. magnifica* x *P. eximia*	New Zealand	Riverlea '86	Name changes to 'Pink Fantasy'
'Riverlea'	*Ls. cordifolium*	New Zealand	Riverlea '86	
'Riverlea'	*Ld. laureolum*	New Zealand	Riverlea '86	Discontinued

Table 4. Cultivar names which are possibly duplications.

Cultivar name	Parentage	Country	Publication
'Cloudbank Ginny'	*Ld.* hybrid	USA	Matthews '92
'Cloudbank Jenny'	*Ld. discolor* x *Ld. gandogeri*	USA	Matthews & Carter '83
'Meteor'	*Ls. lineare* x *Ls. vestitum*	USA	IPA Journal '91
'Meteor'	*Ls. vestitum*	New Zealand	Duncan & Davies '78
'Red Baron'	*P. compacta* x *P. obtusifolia*	South Africa	SAPPEX News '88
'Red Baron'	*P. obtusifolia*	USA	Hawaii Poster '82

Table 5. Member countries of Union for the Protection of New Varieties of Plants (UPOV).

*Australia	France	Japan	*South Africa
Belgium	Germany	Netherlands	*Spain
Canada	Hungary	*New Zealand	Sweden
Czech Republic	Ireland	Norway	Switzerland
Denmark	*Israel	Poland	United Kingdom
Finland	Italy	Slovakia	*United States of America

(* Protea producing countries)

REPRODUCTIVE BIOLOGY OF *BANKSIA*

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Abstract

The genus *Banksia* is characterised by large inflorescences consisting of many individual flowers. The flowers are protandrous and the anthers shed their pollen prior to flower opening on a specialised portion of the style called the pollen presenter. The pollen receptive stigma cells are located within a groove, and do not attain peak receptivity until three days after flower opening, by which time the flower's own pollen has been removed by foraging fauna. The time of peak receptivity of the stigma has been determined experimentally by its ability to support pollen germination, and by the production of stigma secretion. Thus, in the natural situation, protandry is an effective outcrossing mechanism in *Banksia*. In addition to protandry, most species of *Banksia* also show a degree of self incompatibility. Fewer seeds are set following self as compared to cross pollination, and this is due to fewer pollen tubes reaching the base of the style. Some genotype combinations are more successful than others in terms of fertility, and in some cases are more successful when crossed in one direction than in the other. Even when all flowers are cross pollinated, however, seed set is still relatively low. This is partly due to spatial limitations imposed by the size of the infructescence, but is probably also attributable to nutrient allocation within the plant. This limitation in seed set is a problem in *Banksia* breeding, particularly in the production of interspecific hybrids. Nevertheless, some species combinations will produce hybrids, which have potential as new cultivars. The problems involved in studying reproductive biology of *Banksia* is discussed in relation to plant improvement.

1. Introduction

The genus *Banksia* L.f. (Proteaceae) comprises 75 species, nine of which are suitable for cut flower production. The blooms are large and of striking appearance and are used as standards in floral arrangements in similar manner to other proteaceous cut flowers, such as *Protea* species. The *Banksia* genus is native to Australia and most of the world production is sourced from the country of origin. Selection and breeding of banksias is currently underway in Australia, along with research into cultural practices aimed at maximising yield (Sedgley *et al.*, 1991).

2. Floral Structure

Banksia floral structure conforms with the typical proteaceous pattern of large numbers of individual florets grouped together to form conspicuous inflorescences. In *Banksia coccinea* and *B. menziesii* the flowers are produced spirally on the inflorescence, with 13 separate genetic spirals initiating simultaneously (Fuss and

Sedgley, 1990). The flowers develop in pairs, with each flower subtended by a floral bract and the pair of florets and their floral bracts subtended by a common bract (Fuss and Sedgley, 1990). These bracts are inconspicuous in *Banksia*, and the floral display is provided by the coloured perianths and styles. The flower has four tepals, with a single bilobed anther attached by a short filament to the distal region of each. The pistil consists of an ovary, which is embedded in the woody core of the inflorescence, and a long style with a small pollen-receptive stigmatic area in the apical region. During floral development the style elongates more quickly than the perianth and arches beyond the corolla tube by protruding between two perianth members. The distal portion of the style is specialised for pollen presentation, and scanning electron microscopy has shown that its structure varies between species (Sedgley *et al.*, 1993). The receptive stigmatic cells are located in a groove toward the tip of the pollen presenter, which in most species is located longitudinally and obliquely terminal, although in a few it is transverse or lateral. In *Banksia menziesii* the pollen presenter has a complex internal structure as observed by light microscopy, with the transmitting tissue enclosed by transfer cells to maximise water and nutrient supply to the growing pollen tubes (Clifford and Sedgley 1993). The transfer cells are not present in the rest of the style, and the number of transmitting tissue cells declines over the long length of the style. The *Banksia* style is a robust woody structure due to lignified sclerenchyma tissue located in the outer cortex.

The *Banksia* floral display is contributed entirely by the perianth and the style. For example, in *Banksia coccinea*, the inflorescence is grey prior to anthesis due to the grey colour of the perianths, and red following anthesis due to the red colour of the styles.

After flowering, the inflorescence develops into a woody infructescence. Successfully fertilised ovaries develop into follicles embedded in the core of the structure. As the infructescence does not increase in size after flowering, but the mature follicles are much larger than the ovaries at anthesis, it is clear that there are spatial limitations to fertility (Fuss and Sedgley, 1991a,b).

3. Protandry

As in many other proteaceous genera, the anthers of the *Banksia* flower dehisce prior to flower opening and deposit their pollen on the pollen presenter. This generally occurs about one day before the flower opens, and following anthesis the pollen is collected by foraging fauna. At this stage the stigma papilla cells are not receptive to pollen, and peak stigma receptivity occurs at three days after flower opening. This has been measured by increase in the width of the stigmatic groove and by increase in pollen germination on the stigma in *B. menziesii* (Fuss and Sedgley, 1991a), and by increase in stigmatic secretion in *B. coccinea* (Fuss and Sedgley, 1991b). In the natural habitat all of the flower's own pollen has been removed by insects, birds or mammals by the time the stigma is receptive, and the flower may be cross pollinated by a foraging animal which has visited another plant.

4. Self Incompatibility

Most species of *Banksia* which have been studied produce less seed following self pollination than following cross pollination. The self incompatibility is only partial, however, as controlled hand pollination of *B. menziesii* resulted in 80% infructescence

set following crossing compared with 33% following selfing, and 6 follicles per crossed inflorescence compared with 1.3 after selfing (Fuss and Sedgley, 1991c). In *B. coccinea* all pollinated inflorescences set some seed, but the crossed infructescences had 40.7 seeds compared with 27.9 after selfing (Fuss and Sedgley, 1991b,c). Further information was obtained from a 5 x 5 diallel experiment, with the results measured by pollen tube growth, observed using fluorescence microscopy (Fuss and Sedgley, 1991b). Pollen tubes had reached the base of the long style by six days after pollination, but self pollination generally resulted in poorer tube growth. Statistical analysis showed that some plants were more successful parents than others, that some genotype combinations were better than others and that some crosses were more fertile when conducted in one direction than in the other. These results indicate that *Banksia* has a mixed mating system with complex genetic interactions.

5. Hybridisation

Based on knowledge of the reproductive biology of the genus, hybridisation methods have been developed for *Banksia* (Fuss and Sedgley, 1991a). Flowers of known age are identified by removing all open flowers and bagging the inflorescence for 24 hours. After this period all unopened flowers are removed leaving a ring of flowers which have opened within the previous 24 hours. Self pollen is removed from each open flower by moving a looped synthetic pipe cleaner over the pollen presenter part of the style. The bag is replaced, and removed three days later at peak style receptivity. Pollen transfer is achieved by rubbing a pollen-laden pollen presenter from the designated male parent against the stigmatic groove of the recipient pistil. The pollen must be placed inside the groove or it will not germinate. The bag is replace for a few days until the style starts to wither. Using pollen from another plant, 11% follicle set can be achieved with *B. menziesii*. Hybridisation is essential for successful plant breeding, and interspecific hybridisation is the aim of the Australian selection and breeding program (Sedgley *et al.*, 1993).

6. Crop Management

Research into reproductive biology can also be used to improve crop management practices. *Banksia menziesii* and *B. coccinea* both initiate their inflorescences in late spring, but differ in their period of floral development (Fuss and Sedgley, 1990). *B. menziesii* flowers between six and eight months after initiation, whereas *B. coccinea* flowers between nine and twelve months later. This means that the grower has very little time to prune *B. coccinea* bushes after harvest without risking removal of next year's initiated blooms. In contrast, pruning of *B. menziesii* can be conducted over a much longer period without risking loss of the subsequent year's crop. There is also a positive correlation in both species between shoot length, shoot diameter and number of leaves, in relation to the probability of a shoot producing an inflorescence (Fuss *et al.*, 1992). Most blooms of both species are initiated on two year old shoots. Thus time of year, shoot age and shoot thickness and length can be used to assist in the pruning of banksias for optimum production (Sedgley and Fuss, 1992). There is also evidence that site location and climate are important factors in productivity. In *B. coccinea* and *B. menziesii* floral abnormalities were correlated with low temperatures

during the floral development period (Fuss and Sedgley, 1991d, Fuss *et al.*, 1992).

7. Conclusions

Research into reproductive biology of *Banksia* has led to improvements in both breeding and crop management. This will assist in the development of the *Banksia* cut flower industry worldwide. In addition, the research will have benefits for the development of other proteaceous cut flower crops. There are many similarities in reproductive biology across the family, and the research approach as well as some of the findings should be readily applicable to other genera.

Acknowledgements

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DEVELOPMENT OF *LEUCADENDRON* SINGLE STEM CUT FLOWERS

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Abstract

Although leucadendrons are traditionally marketed as greens/foliage-type flowers, a high market demand potentially exists for superior quality *Leucadendron* single stem cut flowers as a separate product. A problem with *Leucadendron* is that the desireable cut flower combination of attractive large flower heads, long flowering branches and a high yield is almost impossible to find within a single species. Therefore interspecific crosses must necessarily be used to combine good qualities from different species. A three-phase programme was launched to breed such leucadendrons: 1) testing for cross compatibility amongst species; 2) an interspecific hybridization programme and 3) selection, and evaluation of the programme. The test study showed that *Leucadendron* species hybridize comparatively well within the sub-genus Alatosperma. In the hybridization programme vigorous, high yielding, F_1 hybrids with exceptionally long stems and attractive, large flower heads were found. In addition these selections have a relatively wide seasonal flowering/marketable period.

1. Introduction

The genus *Leucadendron* is marketed mainly as foliage because of its small flowers and short flowering branches (table 1). Its use as a cut flower is also restricted because of its relatively short flowering season, mainly from June till September/October for the commercial species. An exception is *Leucadendron* 'Safari Sunset'. With its long flowering branches and large, deep pink-red flower heads, it has been one of the most successful international protea cultivars. In the RSA it is marketed out of its natural flowering period mostly during January and February, but it is also attractive and marketable in July, its natural flowering period. Several other cultivars in this class are already in circulation. Thus the commercial value and practicability of single stem *Leucadendron* cut flowers, although limited to a few cultivars, is a well proven fact and a great commercial future potentially awaits the genus.

A possible approach to development is to improve the shortcomings of indivual species by selection of superior types within each species. However a problem with this approach is the serious limitations existing within practically all *Leucadendron* species. For example, the desirable combination of large flower heads, long flower stems and a high yield is very rare within the same species, as in *L. gandogeri* which has large flowers but short flowering branches. The improvement of a species to the status of a single stem cut flower through selection within the species only, is thus impractical. It is therefore clear that crosses must be used to combine favourable qualities from different species. The well-known hybrid ' Safari Sunset' (*L. laureolum x L. salignum*) has both long stems and large flowers as opposed to the parent species which do not have this favourable combination. For this reason it was decided to

initiate an interspecific hybridization programme with *Leucadendron*. A survey of *Leucadendron* species showed a sufficiently wide genetic base available in nature for hybridization of the proposed large flower head and long flower stem parental types.

Heterosis, or hybrid vigour, is a characteristic of the large majority of interspecific crosses in Proteaceae (Brits, 1983). The improvement of production is one of the primary objectives of the hybridization programme. The VOPI had collected a relatively large gene bank of outstanding *Leucadendron* selections over a period of 15 years. These superior within-species clonal selections were available for a proposed hybridization programme.

Another common problem in Proteaceae hybridization programmes is a very low compatibility between species. Considerable work has been done in this area, but little has been published. This interspecific cross compatibility ranges from low to very low in the genera *Leucospermum* and *Protea* (Brits, 1983; Brits & Van den Berg, 1990). In contrast with the other genera, very few natural hybrids have been found in *Leucadendron* (Williams, 1972). To start with, a series of systematic crosses between closely and distantly related *Leucadendron* species was performed, to test the practicability of a full scale hybridization programme.

2. Material and methods

2.1. Testing of cross compatibility in *Leucadendron*

In a diallel hybridization experiment reciprocal crosses were made using plants in a series of decreasing taxonomic relationship (table 2). Progressively greater phylogenetic distance was achieved by crossing between individual plants within ecotype, between ecotypes within species, between species within sub-genus and between sub-genera.

2.1.1. Method

Three different methods of pollination were used.

— Open pollination: Marked flower heads were left uncovered for 3 weeks after anthesis, then covered as below.
— "Passive" pollination: Marked flower heads were prepared for pollination, but were left unpollinated: 20 styles were left on the flower head, the rest dissected out. The flower head was then covered tightly with a glassine bag.
— Controlled cross pollination: Flower heads of 3 species were prepared as in "passive" pollination and the fresh stigmas were then pollinated once with fresh pollen. The ecotypes and species were of decreasing taxonomic relationship (table 2). The combinations are provided in table 2.

Each crossing combination was repeated 5 times (5 flower heads pollinated). Seed was harvested 7 months after pollination and only well developed seeds, sorted by hand, were germinated and counted.

2.2. Hybridization programme

Based on the successes achieved in the study of crossing compatibility, a commercial hybridization programme was launched, using the same methods. The aim of the programme was to improve the weaknessess of *Leucadendron* foliage ("greens") by transferring genes by means of controlled hybridization. Some of the most popular serotinous species, *Leucadendron discolor, L. laureolum* and *L. salignum* were included in the first phase in a series of intra- and interspecies crosses. Specific main breeding aims were set, viz. an increased range of colour forms, larger flowers and long flowering stems (tables 3, 4).

2.3. Selection and evaluation

Seed obtained from the above crossing combinations was germinated. The seedlings were planted in a narrow spacing on the Tygerhoek Experimental Farm at Riviersonderend and grown to maturity. Within the first two seasons putative hybrid plants were identified, marked and cuttings were taken of these. From the third season onward these hybrid cutting plants were evaluated and selections made. Cutting plants of each selection were evaluated over 3 years for flowering period, flower quality, vase life, yield and growth form at the Elsenburg Research Institute. Some of these selections have been marked as candidate cultivars. These will be evaluated commercially in co-operation with leading producers on their farms.

3. Results and discussion

3.1. Testing of crossing compatibility in *Leucadendron*

There was a strong decline in seed set with increasing phylogenetic distance between parents (table 2). For example, *L. discolor* ecotype 1 crossed with *L. discolor* ecotype 2 produced 17 seedlings, the interspecific cross of *L. discolor* ecotype 1 with *L. conicum* yielded 8.6 seedlings, but *L. discolor* ecotype 1 crossed with *L. galpinii* produced no progeny (table 2). Fecundity (seed set) differed strongly within and between species. For example, a) within species, *L. discolor* ecotype 1 produced on average more than twice the progeny of ecotype 2 and b) between species, open pollinated progeny differed largely (table 2). Poor compatibility was found between taxonomic sections, e.g. *L. galpinii* produced almost no progeny in crosses with the section Alatosperma (table 2).

3.2. Hybridization programme

More than 3 000 seedlings were obtained from c. 8 500 individual pollinations in a total of 36 cross combinations within and between species (table 5). Seed set was almost 50% of florets pollinated. These results are relatively good compared with hybridization success in *Leucospermum* and *Protea*.

Early inspection showed that the majority of seedlings were indeed crosses as they represented intermediate forms. Examples of individual crosses made and results are presented (table 5). Although these results vary markedly, the overall efficiency

of crossing was high.

3.3. Selection and evaluation

At least 9 different cross combinations have yielded promising hybrids (table 6). These plants constitute a considerable variety of new flower head shapes. Both male and female plants show promise for marketing. The flower head sizes vary from large (6 — 12 cm) to extra large (12 — 18 cm), the latter group comprising c. one-third of these selections. The stem lengths of these selections are generally in the required cut flower bracket. Some selections produce a reasonable crop of exceptionally long stems of up to 100 cm. The flowering/marketable period of these selections are spread from June to September (table 6).

Conclusions

It is clear from the results that the production of progeny between *Leucadendron* species of the section Alatosperma is much higher than in the case of *Leucospermum* and *Protea*. The compatibility between the two *Leucadendron* taxonomic sub-genera is however of the same low order as it is with *Leucospermum* and *Protea*. Fecundity differs within and between species. Thus cross compatibility is positively correlated with relationship.

The aims set in the hybridization programme have been met satisfactorily. Vigorous F_1 hybrids with exceptionally long stems and attractive large flower heads were obtained. High yield in these plants results from strong F_1 hybrid vigour. In addition these selections flower over a relatively wide seasonal period. The stage is thus set for future hybridization programmes in *Leucadendron*. The present collection of superior F_1 hybrid selections should contribute meaningfully to a new protea product, *Leucadendron* single stem cut flowers, of which 'Safari Sunset' was the forerunner.

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Table 1. Estimated value of fynbos exported 1992.

CATEGORY	RAND M
Leucadendron cut flowers	c. 500
Other protea cut flowers	c. 8 000
Leucadendron foliage	c. 4 000
Other-type foliage	c. 7 325
Dried flowers	c. 20 000

Table 2. Mean seedling numbers recruited per seed head (and % seed set); 20 florets on 5 flower heads pollinated per cross, using *Leucadendron* species and ecotypes of different affinities.

		♀ Parent			
♂ Parent	Eco-type	*L. disc.* a-ecotype 1	*L. disc.* b-ecotype 2	*L. conicum*	*L. galpinii*
1. *L. discolor*	1	3.3	17.0	8.6	0.0
2. *L. discolor*	1	3.8	10.0	4.0	0.2
3. *L. discolor*	2	3.8	4.8	1.8	0.0
4. *L. discolor*	2	4.8	6.2	7.2	0.0
L. conicum	—	1.3	0.0	12.2	0.0
MEAN		3.4 (23)	7.6 (51)	6.8 (45)	0.04 (0.3)
Passive ⊗		0.0	0.0	—	0.0
Open-poll.		20.2 (30)	14.4 (21)	30.2 (52)	0.6 (1)

Table 3. Salient characters of some ♂-parents used in *Leucadendron* interspecies hybridising programme.

Identity		Size of flower-head	Stem-length (cm)	Flowering period	Colour of flower-head	Shape of flower-head/ others
Code	Species/ variant					
T86 08 03	*L. discolor*/ Ecotype 1	Medium	30 - 40	Sept. - October	Yellow	Tulip/Big red 'pom-pom'
T86 09 17	*L. discolor*/ Kommetjie	Large	40	Sept. - October	Light yellow	Tulip/Double involucre
'Duet'	*L. salignum* x *L. sessile*	Medium	40	June	Yellow and deep pink-red	Small foliage leaves
T89 08 10	*L. eucalyptifolium*	Large	30 - 60	August	Deep yellow	Vigorous, high yield
T81 07 19	*L. elimense* subsp. *salterii*	Large	40 - 70	July	Light yellow	Daisy/Small foliage leaves

Table 4. Salient characters of some ♀-parents used in *Leucadendron* interspecies hybridising programme.

Identity		Size of flower-head	Stem length in cm	Flowering period	Colour of flower-head	Shape of flower-head/ others
Code	Species/ variant					
T74 03 07	*L. salignum* Langkloof	Large	40	June-July	Yellow. End Jan. deep red	Rosebud/ *Phytophthora c.* resistant
T81 07 03	*L. salignum* Helderfontein	Medium	30 - 60	June/July	Light yellow	Rosebud
T82 11 56	*L. salignum* Buffelsnek	Large	60 - 70	End of June-July	Light yellow-green	Rosebud/ Vigorous
T86 08 02	*L. salignum*	Large	30	August	Light yellow	Open wider
'Safari Sunset'	*L. laureolum* x *L. salignum*	Large	40 - 60	July	Yellow and pink-red	January-February
T81 07 01	*L. laureolum*	Large	40	June-July	Yellow-white	Tulip/Vigorous
T86 09 02	*L. laureolum*	Large	30 - 40	August-September	Yellow-green	Tulip
T86 09 05	L. laureolum	Medium	40	September	Yellow-green	Upright growth habit

Table 5. Efficency of *Leucadendron* breeding programme: example representing hybridization results.

Code	Species	No of flower-heads pollinated	No of florets	Seed harvested (% of florets)	Seedlings (% germination)
T93 06 06	*L. salignum* x *L. discolor*	10	300	270 (90)	34 (13)
T93 07 36	*L. laureolum* x *L. sal. x L. sessile*	2	30	6 (20)	6 (100)
T92 07 18	*L. laureolum* x *L. discolor*	2	90	77 (85)	26 (34)
T93 08 60	*L. laureolum* x *L. discolor*	3	105	87 (83)	37 (42)
T93 07 31	*L salignum* x *L. sal. x L. sessile*	5	125	94 (75)	11 (12)
T93 08 10	*L. laureolum* x *L. eucalyptifolium*	14	420	375 (89)	375 (100)
T93 08 02	*L. laureolum* x *L. elimense*	13	455	393 (86)	126 (32)
TOTALS FOR PROGRAMME:					
Crossing combinations: 36		203	8 475	3 957 (47)	3 166 (80)

Table 6. Sample selections made from F_1 hybrids in *Leucadendron* interspecies hybridization programme. Flower-head sizes (cm): extra large, 12 — 18; large 6 — 12; medium 3 — 6 and small < 3.

Code	Parent species; (hybrid parent)	♂/♀	Size of flower-head	Stem length in cm	Flowering and marketable period*	Colour of flower-head
T92 07 07	*L. salignum x L. eucalyptifolium*	♂	Large	60	July	Deep yellow
T92 07 08	*L. coniferum x L. eucalyptifolium*	♀	Large	40 - 60	July	Yellow-green
T93 08 02	*L. laureolum x L. elimense*	♀	Extra large	55	August September*	Yellow-green and red
T92 07 18	*L. laureolum x L. discolor*	♀	Large	40	July	Light and dark yellow
T93 06 06	*L. salignum x L. discolor*	♀	Large	40	July May - Sept*	Yellow-white pink-red
T93 07 31	*L. laureolum x (L. sal. x L. sessile)*	♀	Large	40 - 50	July	Yellow-green
T93 07 36	*L. salignum x (L. sal. x L. sessile)*	♂	Medium	45	July	Pink-red and yellow
T93 08 10	*L. laureolum x L. eucalyptifolium*	♂	Extra large	40 - 60	August	Light yellow
T93 08 60	*L. laureolum x L. discolor*	♀	Extra large	40	August-September	Yellow-green and pink-red

PRELIMINARY RESULTS ON FACTORS AFFECTING *IN VITRO* GERMINATION AND STORAGE OF *PROTEA* POLLEN

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Abstract

The effects of pH, sucrose, boric acid and temperature upon *in vitro* germination of *Protea repens* (L.) L. cv. Embers pollen were investigated in hanging-drop culture to optimize conditions for germination. The basal medium consisted of 300 ppm $Ca(NO_3)_2.4H_2O$, 200 ppm $MgSO_4.7H_2O$ and 100 ppm KNO_3 in distilled water. The following ranges of the variables tested were found to be optimal for fresh pollen: pH (5 — 8), sucrose concentration (0.4 to 0.7M), boric acid concentration (50 to 500 ppm) and incubation temperature (5 — 30 °C). There were no significant differences in germination percentage or pollen tube length within these ranges, but a sharp drop in germination occurred beyond these ranges. In all cases pollen tubes did not reach a length of more than 120 μm.

The influence of storage temperature and humidity on pollen viability were studied in two *Protea* clones. Pollen was stored at a range of temperatures and relative humidities (r.h.) for up to 6 months and tested for ability to germinate *in vitro* following a period of hydration at high humidity. Pollen stored at -196 °C in liquid nitrogen and at -14 to -18 °C in a deep freezer, retained a germination percentage as high as that of fresh pollen (> 90%). Pollen stored at 2 to 7 °C and 22 to 27 °C maintained a moderate (> 50%) germination percentage for 75 and 30 days respectively at the 10 and 30% r.h., while the germination percentages dropped much quicker at the 60% r.h.

1. Introduction

Genetic improvement in the genus *Protea* is hampered by a lack of breeding technology. Severe bottlenecks include inability to hybridize between species, low hybrid seed set and lack of pollen storage techniques (Brits, 1984). The maintenance of pollen viability facilitates the crossing of plants which flower at different times. It would therefore be useful if the pollen could be kept viable from one season to the next or longer. Temperature and relative humidity are the major factors affecting pollen storage in many species (Stanley and Linskens, 1974; Visser, 1955). Long-term storage of pollen, to be used in breeding projects, necessitates monitoring of pollen viability, but techniques for this have not been fully developed for *Protea*. The viability of fresh and stored pollen is best determined by germination tests (Stanley and Linskens, 1974). In preliminary investigations the Brewbaker and Kwack (1963) medium produced excellent germination of fresh *Protea* pollen (> 90%) but pollen tubes did not reach a length of more than 120 μm before bursting.

The present study was undertaken to determine the optimal germination medium and storage conditions for *Protea* pollen and this paper reports on the progress being made towards meeting this objective.

2. Materials and methods

2.1. Pollen collection

All the experiments were conducted during 1992 and 1993 on three clones planted in experimental plantations at Elsenburg (33°50' S, 18°51' E) and Riviersonderend (34°09' S, 19°54' E) in South Africa. Prior to the experimental period all plants had been subjected to routine plantation management practices, including drip-irrigation during the summer months. Harvesting of blooms in previous years served as the only form of pruning of the bushes. Pollen of *Protea repens* (L.) L. cv. Embers was used in the germination experiments. Five inflorescenses from five different Embers plants, with approximately one-quarter of the florets having undergone anthesis, were harvested and brought to the laboratory where the stems were placed in water. All the open florets were removed and 16 hours later all the florets that had opened in the meantime were harvested (± 25 per inflorescence). The pollen was scraped off and thoroughly mixed. The pollen mixture was immediately used to draw out five replicates for each treatment and this procedure was followed for all the germination experiments.

For the storage experiments pollen of *Protea magnifica* Link. clone T84 07 05 and *Protea eximia* (Salisb. ex Knight) Fourcade cv. Fiery Duchess were used. Five inflorescenses per plant from five plants were used and handled in the same way as previously described, with the exception that this time the pollen from each plant (five inflorescenses) was mixed and used as a replicate. All the replicates were first dried for 24 hours in a desiccator over silica gel at 5 °C, whereafter the pollen mixtures were divided into the different samples and placed in gelatine capsules for storage.

2.2. Pollen germination

The *in vitro* germination of the pollen was carried out over a period of five weeks, commencing in the middle of the flowering season of cv. Embers. The capacity of the pollen to germinate and to produce normal pollen tubes was tested by the hanging drop technique of Van Tieghem (1869) using an artificial sucrose growth medium. Preliminary germination tests established no significant differences among $Ca(NO_3)_2.4H_2O$, $MgSO_4.7H_2O$ and KNO_3 concentrations on germination or pollen tube growth of *Protea* pollen, so these were not included in the tests. In efforts to optimize the composition of the medium for germination of *Protea* pollen, pH from 2 to 11, sucrose concentrations of 0 to 1.0 M, H_3BO_3 from 0 to 1 000 ppm and incubation temperatures from 5° to 40 °C were tested. The basal medium consisted of 300 ppm $Ca(NO_3)_2.4H_2O$, 200 ppm $MgSO_4.7H_2O$, 100 ppm KNO_3 in distilled water. In all experiments except those involving changes in sucrose concentration, the sucrose concentration was adjusted to 0.5 M and in all experiments except that on pH effect, the pH was adjusted to 7.0 using 0.1 M HCl or KOH solutions. Since germination occurred within a short period of time, it was scored after three hours using a microscope at a magnification of x 200. A minimum of 200 randomly selected pollen grains in four different fields were scored for germination and only pollen grains producing tubes longer than the grain diameter were counted as germinated. All treatments were replicated five times.

2.3. Pollen storage

The gelatine capsules with pollen samples were stored at room temperature of 22 to 27 °C, in a household refrigerator at 2 to 7 °C, in a household freezer at -14 to -18 °C and in liquid nitrogen at -196 °C. At all the temperatures, except liquid nitrogen which has negligible water vapour pressure, the pollen samples were stored at relative humidities (r.h.) of 10, 30 and 60%. The humidities were maintained using different concentrations of sulphuric acid (Young, 1967; Solomon, 1951). The atmospheres were produced in closed 1 000 mℓ flasks and were allowed to equilibriate for at least one month before use. For storage at -196 °C, the pollen was placed in 5 cm^3 plastic cryovials which was directly submerged in liquid nitrogen in a nitrogen storage vessel. No cryoprotectant was used to treat pollen before freezing. Each replicate was scored for pollen germinability before storage. All frozen samples were thawed for three hours at 25 °C in a 100% r.h. atmosphere before testing. Pollen stored at room temperature was tested for germinability every 15 days while the other treatments were tested after 30, 90 and 180 days of storage. The germination medium used in the storage experiments was identical to the previous mentioned basal medium with 100 ppm H_3BO_3 and a sucrose concentration of 0.4 M. Pollen germination percentages were determined in the same manner as previously described.

2.4. Statistical analysis

Each germination experiment was laid out in a completely randomized design and the data were analyzed by means of a one-way analysis of variance. The two clones in the storage experiments were investigated in separate trials, and each trial was laid out in a completely randomized, 2 x 3 factorial design. Five replicates were involved in each trial. All significance tests and least significant differences (LSD's) were computed at the 5% level of probability.

3. Results

3.1. Pollen germination

Germination of the pollen commenced within 15 minutes after inoculation and was completed within 2 hours whereafter the pollen tube ends ruptured and the contents exuded. The three species tested did not differ significant in their capacity for pollen tube elongation and in all cases pollen tubes did not reach a length of more than 120 μm.

There were no statistical differences between results of the pH 5 to 7 treatments (figure 1). A pH value of 7.0 was used in the ensuing experiments. The optimum sucrose concentration range for pollen germination was between 0.4 M and 0.7 M, with no statistical differences between the 0.5 and 0.6 M treatments (figure 2). Very few pollen grains germinated at 0.0 M sucrose, but germination increased significantly when sucrose concentration was increased. A sucrose level higher than 0.7 M inhibited germination as well as pollen tube growth. A sucrose concentration of 0.5 M was used in the ensuing experiments. Boric acid levels from 50 — 500 ppm were optimal with no statistical differences between the 50, 100 and 200 levels (figure 3). With no boric acid

in the medium a significant percentage of pollen still germinated (32.8%) but the majority of pollen grains only formed short protuberances and were therefore not counted as germinated. A higher level of boric acid (> 500 ppm) was slightly inhibitory to germination as well as to pollen tube growth. A boric acid concentration of 100 ppm was used in the ensuing experiments. Pollen germination was maximal at a very wide incubation temperature range of 5 — 30 °C with no statistical difference between the 15, 20 and 25 °C treatments (figure 4). A marked drop in germination occurred beyond 30 °C with no germination occuring at 40 °C. An incubation temperature of 25 °C was used in ensuing experiments.

3.2. Pollen storage

Pollen of both clones stored at -196 °C in liquid nitrogen (figure 5) and at -14 to -18 °C in a freezer (figures 6, 7) retained a germination percentage as high as that of fresh pollen (> 90%) after 6 months. There were also no statistical differences between the three r.h. treatments at the -14 to -18 °C storage temperature for both clones tested. Pollen stored at the 2 — 7 °C temperature maintained a germination percentage as high as that of fresh pollen for only one month for both clones followed by a sharp, statistically significant, decline in germinability (figures 8, 9). In contrast to the lower temperature treatments, a statistically significant difference was observed between the three r.h. treatments. The 60% r.h. treatments showed the most rapid decline while the 30% r.h. treatments showed the best germinability after three months, but at all three r.h. treatments, germinability was almost nil after 6 months with only the 30% r.h. treatment of *P. eximia* statistically higher than 0%. Pollen germination was particulary poor in all the samples stored at room temperature (22 to 27 °C) with almost 0% germinability at the 60% r.h. treatments for both clones after just 15 days of storage (figures 10, 11). The situation with the other two r.h. treatments was significantly better but no sample had any germinability after 90 days of storage.

4. Discussion

Although a complete study of all the factors that could influence pollen germination was not included in this study, the extremely high germinability (> 90%) obtained with *Protea* pollen suggests that the medium used in this study could be very close to the optimal medium despite the short pollen tubes obtained. *Protea* pollen grains are quite small, ± 30 μm in diameter depending on species, and it appears that the pollen grain's own resources to produce and sustain pollen tube growth are very limited and that the pollen needs some sort of nourishment from the style for it to produce longer tubes. This must be further investigated. Lee, Thomas and Buchmann (1985) found that jojoba pollen germinated poorly under a temperature lower than 15 °C, but *Protea* pollen showed no such tendency although it took slightly longer to germinate and pollen tube growth was a little slower. Since Schmucker (1935) demonstrated the essential role of boric acid on pollen germination, many investigators (Brewbaker and Kwack, 1963; Vasil, 1964; De Bruyn, 1966; Dickinson, 1978; Visser, 1955) confirmed this for various plants. This study showed that although some germination (32.8%) still occurred without the addition of boric acid, a small amount of boric acid is essential to obtain good pollen germination in *Protea*. Considering all the facts in the present study

the following medium has been chosen as optimal for *Protea* pollen: 300 ppm $Ca(NO_3)_2$. $4H_2O$, 200 ppm $MgSO_4.7H_2O$, 100 ppm KNO_3, 100 ppm H_3BO_3, 0.5 M sucrose, pH 7.0 and an incubation temperature of 25 °C.

Protea pollen could be stored for at least six months in liquid nitrogen and in a freezer and still produce germinability equal to that of fresh pollen. Theoretically pollen stored in liquid nitrogen should retain its viability idefinitely (Stanley and Linskens, 1974; Sedgley, 1981) provided precautions are taken to reduce pollen moisture before storage (Yates *et al.*, 1991). For crossing purposes freezer storage appears the most practical and economic means of storing *Protea* pollen. Further results in this study should indicate if the same situation exists after a full year of storage. The present study showed that long term storage of *Protea* pollen is not feasible at temperatures above zero. Many investigators (Stanley and Linskens, 1974; Griggs *et al.*, 1981) have shown that germinability obtained does not always correspond well to ovule penetration, the ultimate test of pollen viability, and further work should be doen to measure this relationship in *Protea*. Our results suggest, therefore that it may be possible to store pollen of many, if not all *Protea* species relatively cheaply in an ordinary household freezer without any humidity control for long enough to suit the breeding requirements.

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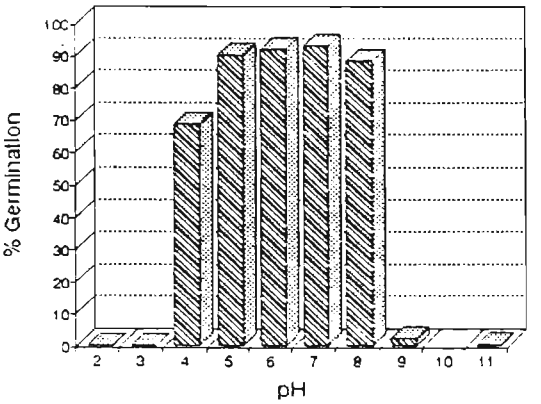

Figure 1- The effect of pH of the medium on *in vitro* germination of protea pollen. LSD(5%)=5.32

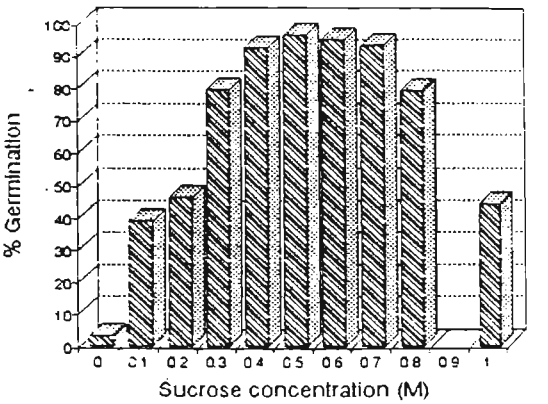

Figure 2 - The effect of sucrose concentration of the medium on *in vitro* germination of protea pollen. LSD(5%)=10.52

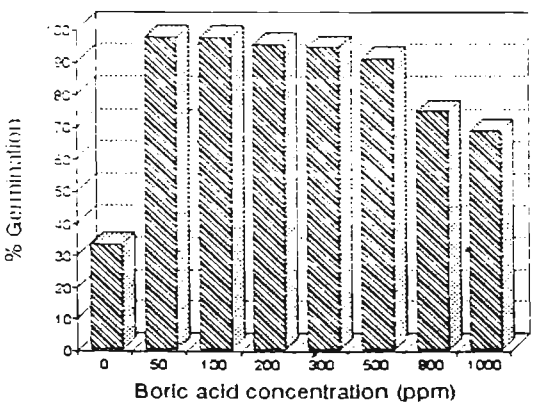

Figure 3 - The effect of boric acid concentration of the medium on *in vitro* germination of protea pollen. LSD(5%)=9.43

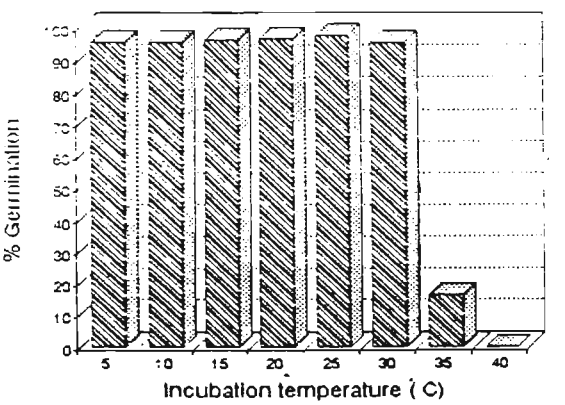

Figure 4 - The effect of incubation temperature on *in vitro* germination of protea pollen. LSD(5%)=3.61

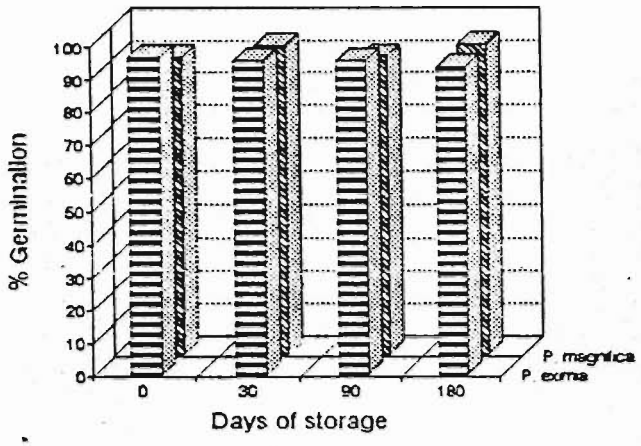

Figure 5 - The effect of liquid nitrogen storage on pollen germination of *P. magnifica* and *P. eximia*

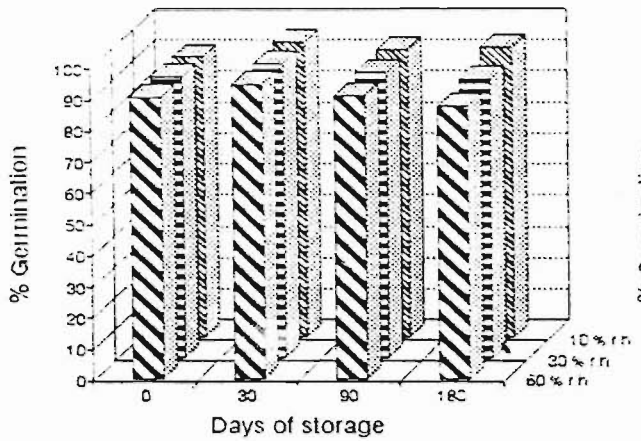

Figure 6 - The effect of freezer storage at different relative humidities on pollen germination of *P. magnifica*

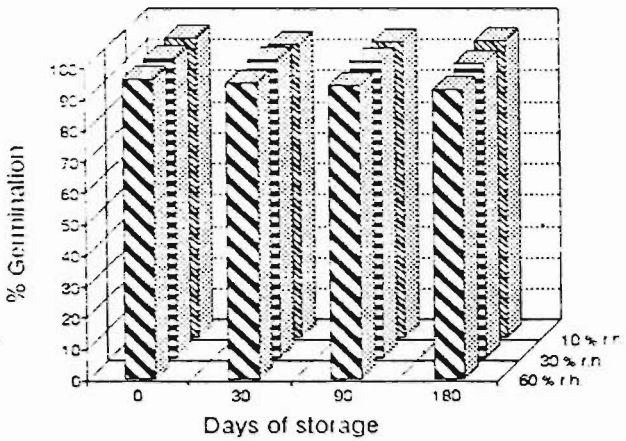

Figure 7 - The effect of freezer storage at different relative humidities on pollen germination of *P. eximia*

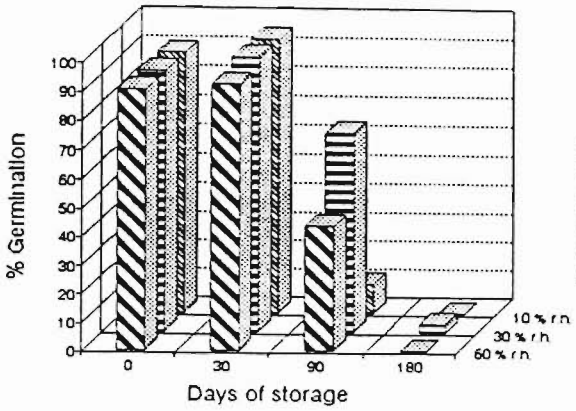

Figure 8 - The effect of refrigerator storage at different relative humidities on pollen germination of *P. magnifica*

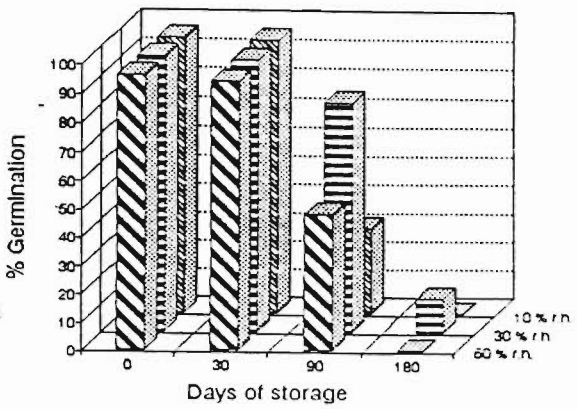

Figure 9 - The effect of refrigerator storage at different relative humidities on pollen germination of *P. eximia*

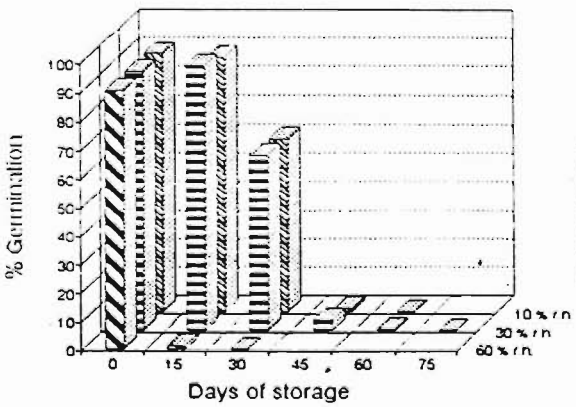

Figure 10 - The effect of room temperature storage at different relative humidities on pollen germination of *P. magnifica*

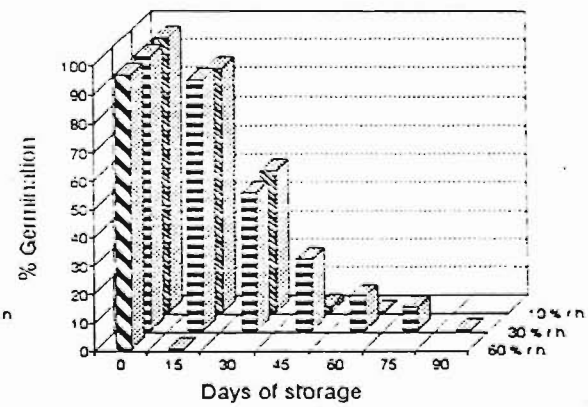

Figure 11 - The effect of room temperature storage at different relative humidities on pollen germination of *P. eximia*

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