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Second International Protea Research Symposium

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R.A. Criley

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SECOND INTERNATIONAL PROTEA RESEARCH SYMPOSIUM

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Editor

Dr. Richard A. Criley

Convener

Dr. Philip E. Parvin

Section for Ornamental Plants

Protea Working Group

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PREFACE

The Second International Protea Research Symposium was organized in San Diego, California, USA, to coincide with the 5th International Protea Conference of the International Protea Association. The gathering of growers and scientists proved to be an excellent opportunity for the exchange of ideas, and it set the stage for future joint meetings.

The publication of these papers in this volume of Acta Horticulturae was made possible by a generous contribution from the organizers of the IPA Conference and a grant from the Fred C. Gloeckner Foundation of New York City, NY.

As convenor of the research symposium, I wish to thank Mr. Dennis Perry, President of IPA and Chairman of the protea conference; Mr. Brian Young, Consul (Agricultural Affairs), South African Consulate-General, Los Angeles, who took on the responsibility for organizing and staging the poster presentations; and Dr. Richard A. Criley of the Horticulture Department of the University of Hawaii, who not only was of great assistance in organizing the symposium, but agreed to take on the task of editing these proceedings.

It is with sorrow that we note that Dr. Tok Furuta, University of California, Riverside, who had begun the organizing of the symposium, passed away after a short illness soon thereafter.

To Mr. Gert Brits who succeeds me as Chair of the Protea Working Group of the International Society for Horticultural Science, I extend best wishes as he leads the activities of the working group during the next quadrennium.

Philip E. Parvin
Convenor

ROOTSTOCK PRODUCTION RESEARCH IN *LEUCOSPERMUM* AND *PROTEA*: I. TECHNIQUES

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Abstract

The problems of ineffective conventional grafting and budding techniques were studied. Wedge grafting with elastic PVC tape gave the best results in uniting and securing the scion to the rootstock, respectively. Controlling graft scion desiccation by reducing scion size to two buds, subtended by 1/2 cm^2 leaf sections, eliminated the need for an intermittent misting system required by the conventional method. This allowed the development of new handling methods of rootstocks viz. cutting grafting, grafting of containerised rootstocks under 50% shade, and topworking of young rootstock plants in the field. A modified chip budding technique was successfully tested. Autumn is the best season for grafting to effectively produce new scion growth prior to establishment in the field. These methods, suitable for rapid large-scale commercial production of grafted/budded proteas on clonal rootstocks, compare favourably with the rooting of cuttings in many cases.

1. Introduction

Historically the economic growth of pincushion (*Leucospermum* R.Br.) and *Protea* L. cut flowers has been curtailed by severe restrictions in their cultivation potential. Their strict nutritional requirements are, for example, well-known: low pH-soils, very low conductivity of the soil solution (Vogts, 1980), low levels of certain elements eg. K and especially P and, in many species, a strong preference for NH_4 over NO_3 nitrogen (Claassens, 1986). The ideal soils for these proteas are comparatively rare. Thus fixed adaptations to the narrow nutritional amplitude of the plants in nature severely limits their cultivation outside their natural habitat.

The grafting of proteas on lime-tolerant rootstocks is regarded as economically feasible in Israel. A research programme has resently been launched to develop protea rootstocks for its predominantly calcareous soils (J. Ben-Jaacov, personal communication).

Both pincushions and *Protea* are relatively difficult to propagate by vegetative means. Rooting of the woody cuttings

of *Protea* are especially problematical, many species yielding low rooting percentages even after extended periods in the mist propagation frame (Gouws, 1978; Brits, unpublished data). Thus the most promising cultivars can often be multiplied only at a very slow rate, restricting their cultivation and marketing potential.

The grafting of superior cultivars of woody plants which are difficult-to-root on easily produced rootstocks is a standard practice in commercial horticulture (Hartmann & Kester, 1983). Budding, which makes more economical use of propagating material than grafting, could especially be effective in the multiplication of difficult-to-root and slow-growing cultivars of *Protea* (eg. Harrington, 1988).

Soil-borne pests and diseases present critically important problems to protea growing in many production regions. The genus *Leucospermum* is extremely susceptible to root rot caused by *Phytophthora cinnamomi* Rands. Average losses in affected pincushion "orchards" in South Africa can amount to more than 25% p.a., even on well-drained sites, forcing premature abandonment of such plantings (Von Broembsen & Brits, 1985). In some areas of both South Africa and Australia *P. cinnamomi* is naturally distributed in the indigenous vegetation (Von Broembsen, 1983; Von Broembsen & Kruger, 1984; Cho, 1983) which presents a source of continuous infestation to cultivated proteas. Nematode attack is another serious problem of cultivated *Protea* and *Leucospermum* species, especially in the U.S.A. (Rohrbach, 1983). Thus in many areas the unusual disease risks involved in protea production discourage industrial growth.

Considerable research inputs have been made recently in Australia to develop *Banksia* (Proteaceae) rootstocks tolerant to *Phytophthora cinnamomi* (McCredie et al, 1985). Many of the commercially produced *Banksia* species are, as in the case of *Leucospermum*, extremely susceptible to *Phytophthora* root rot (Cho, 1981). A slight degree of genetic tolerance against *Phytophthora* is apparently present in cut flower pincushions, as well as in the genus *Leucospermum* as a whole. Selection and development of relatively tolerant rootstocks within the genus *Leucospermum* is therefore indicated as a promising short and medium term approach to the root rot problem.

A host of cultivation problems are clearly associated with the specialized root systems of proteas. This situation invites the commercial introduction of grafted proteas on superior rootstocks - as is the case with almost all horticultural crops with a similar growth habit.

Grafting technique

An important impediment in the development of a rootstock

production system for proteas is the lack of commercially suitable grafting and budding tecniques. The standard method for grafting is wedge grafting of semi-hardwood shoot sections on seedling rootstocks whilst retaining complete leaves on the scion. Grafting consequently demands an intermittent misting system for wound healing (Rousseau, 1966; Vogts & Rousseau, 1976). This production system is relatively cost ineffective and consequently a need exists for rapid grafting techniques of selected, vegetatively multiplied rootstocks. The revaluation of techniques in conjunction with the development and release of special rootstock cultivars are therefore indicated.

2. Materials and methods

Approximately 40 grafting and budding experiments with *Leucospermum* and *Protea* were conducted between 1976 and 1980. Aspects nvestigated included improved production and handling methods of rootstocks, grafting and budding techniques, the role of season of the year and evaluation of the relative effectiveness of grafting. Treatments were repeated and combined in different experiments. These were further repeated in different seasons of the year. Of the variables investigated and rootstocks tested only the most important are summarized below. Some of the experiments illustrating pertinent procedures are described in detail.

2.1. Production and handling methods of rootstocks

The grafting and budding of cuttings of selected rootstock clones were compared with the standard use of seedling rootstocks (Vogts & Rousseau, 1976). Time required to produce the rootstock, uniformity of material and consistency of results were considered.

Three methods of handling the rootstock were developed for both grafting and budding. The improved techniques for grafting and budding, referred to below, were used in these experiments.

- As a control, 6-12 month old rooted, containerised rootstock cuttings were grafted and budded in a lathe house nursery (50% shade) without using a misting system.
- Cutting grafting (Hartmann & Kester, 1983). Proteas were grafted/budded on unrooted rootstock cuttings of selected clones whilst being rooted in a standard rooting frame for proteas (Jacobs & Steenkamp, 1976) at the same time. This technique was used successfully for rhododendrons (Eichelser, 1967) and can result in greatly reduced time and space needed to produce grafted/budded plants.
- Topworking. In a third treatment established rooted rootstocks were grafted and budded <u>in situ</u> (Hartmann & Kester, 1983). These rootstocks were always chosen young

i.e. plants were topworked within two years of establishment and within 30 - 45 cm from ground level.

2.2. Grafting techniques

Several grafting techniques and variations of the standard wedge grafting technique were tested.

2.2.1. Reducing desiccation of the scion.

Elimination of an intermittent misting system for large-scale commercial grafting requires a much smaller total leaf area of the scion than in conventional grafting. Two procedures were tested to achieve this: a) reducing the number of buds on the scion, and thus the number of leaves, and b) reducing the size of the subtending leaf of each bud.

Additional methods tested to reduce desiccation of the scion under lathe house and field conditions involved the inclusion of the scion in a) transparent polyethylene plastic, b) blue (infra-red absorbing) polyethylene plastic of the "banana sleeve" type and c) spraying the scion with anti-transpirants.

2.2.2. Securing the scion to the rootstock

Different methods of securing the scion and rootstock were compared. These included a) covering the union with hot grafting wax and b) the use of various types of graft wrapping materials. The main objective of these tests were to reduce the aftercare required of successfully grafted plants.

The usefulness of "Steri-crepe", an elastic, adhesive crepe rubber tape was tested extensively for this purpose since it decomposes naturally and slowly. This could possibly eliminate the need for unwrapping the scion following wound healing and growth resumption. Test results indicated that "Steri-crepe" decomposed too rapidly for use on proteas. Painting the "Steri-crepe" with wound sealant was consequently tested in an attempt to slow down the rate of perishing of the rubber.

The following experiment illustrates the comparison of four methods of securing the scion and rootstock and reducing desiccation of the scion. 237 scions of 18 *L. cordifolium* clones selected from the same genetic stock were wedge grafted on unrooted *L. patersonii* stem cuttings in autumn. The four treatments were:

1. Wrapping the graft union with standard elastic PVC grafting tape, scion left open ("control");
2. P.V.C. grafting tape used, scion covered with closely fitting blue (banana sleeve type) polyethylene plastic;
3. Wrapping the graft union with "Steri-crepe", both scion

and graft union covered with blue plastic;
4. Wrapping the graft union with "Steri-crepe" followed by painting the rubber wrapping with blue PVC-based wound sealant.

Grafted rootstock cuttings were terminal shoot sections of the current season's growth, 25-30 cm in length and of the semi-hardwood type. Scions were cut with two buds and each subtending leaf was reduced to 0,5 cm^2 area. Scions were secured with elastic PVC tape. Leaves were retained on 8-10 cm of the rootstock cutting below the graft. Leaves were stripped below this area and, following grafting, the cutting was given a fresh cut 8-10 cm below its leafy section, followed by basal dips in 0,5% IBA solution and a fungicidal powder (Brits, 1986). Cuttings were planted in a standard rooting mixture for proteas in plastic propagating trays and placed in a bottom heated rooting frame (23°C) in a lathe house under 50% shade (Brits, 1986). Newly grafted scions were sprayed with a mixture of benomyl and captab and allowed to dry; a programme of weekly sprays of exposed scions was maintained until the first signs of new scion growth appeared.

Since the clonal scion population was genetically relatively uniform and a single rootstock was used, one half of the clones was allocated to treatments 1 and 2 and the other half to treatments 3 and 4. Treatments were completely randomized using 7 replications for treatments 1,2 and 11 replications for treatments 3,4. Propagating trays were rerandomized on a daily basis to minimize the effect of uneven heating. Cuttings were scored for adventitious root development and the survival of scions observed, at 6 and 12 weeks. Rooting scores were based on an ordered scale (Snedecor & Cochran, 1967) of 2 = transplantable (Jacobs & Steenkamp, 1976); 1 = roots present but not transplantable; 0 = no roots. The sum of 6 and 12 week scores were expressed as a percentage of the potential maximum score. The results were analysed as attribute data (Snedecor & Cochran, 1967).

Transplantable cuttings were immediately lifted and planted in 2l containers in a lathe house nursery providing 50% shade. Survival and resumption of growth of scions on transplanted cuttings were observed during the ensuing spring at six months from rooting date.

2.2.3. Uniting the scion and rootstock.

Wedge, whip, root and approach grafting (Hartmann & Kester, 1983) were evaluated. The following factorial experiment illustrates the comparison of approach and wedge grafting using four rootstocks. The main objective was to test approach grafting as a method to a) reduce the high percentage losses found in grafted *Protea* due to wound blackening in both the scion and rootstock and b) increase the efficiency of propagating both *Protea* and *Leucospermum*

clones which are difficult to root. Approach grafting is commonly used to successfully unite plants which are difficult to graft together otherwise (Hartmann & Kester, 1983). *P. magnifica* is known to root with relative difficulty and its leaves blacken easily whilst *L. cordifolium* cv. Vlam gives a relatively low rooting percentage (Brits, 1986). Rootstocks were selected for a) vigour, b) rootability and c) compatibility with scions.

The experiment was repeated over two years; only the results of the second year are presented. Unrooted rootstock cuttings were grafted in autumn and the success of the two cutting grafting procedures were compared with the rooting performance of cuttings of the four ungrafted rootstocks as well as the four scion selections. The four treatments were thus:

1. Rootstock cuttings approach grafted by the tongued method (Hartmann & Kester, 1983);
2. Rootstocks wedge grafted;
3. Non-grafted rootstock cuttings;
4. Cuttings of scion selections.

Four scion-rootstock combinations, two each of *Leucospermum* and *Protea*, were used:

Rootstock:	Scion:
1. *Leucospermum tottum* x *L. formosum* (F_1) (T75 11 02)	*L. cordifolium* "Vlam"
2. *L. patersonii* x *L. cordifolium* (F_1) (T76 01 09)	*L. cordifolium* "Yellow Bird"
3. *Protea eximia* Rootstock 78/19	*P. magnifica* - Koo variant
4. *P. eximia* Rootstock 78/18	*P. aristata* (T74 12 05)

Grafted rootstock cuttings were of the same type and length as described above (2.2.2). Non-grafted rootstock and scion cuttings were 18-20 cm terminal shoot sections. Scions were cut with two buds and each subtending leaf was reduced to 0,5 cm^2 area. Scions were secured with elastic PVC tape. Leaves were retained on 8-10 cm of the rootstock cutting below the wedge graft or on the upper section of approach grafted and non-grafted cuttings. Cuttings were stripped of leaves on their lower half and treated basally with IBA and fungicidal powder; all cuttings were planted in a standard rooting mixture for proteas as before (2.2.2). Five cuttings were used per replicate with four replicates per treatment. Treatments were completely randomized. Rootstock cuttings were scored for adventitious root development and surviving scions at 6 and 12 weeks (3 months) for *Leucospermum*, and 12 and 22 weeks (5 months) for *Protea*. Rooting scores were calculated as above (see 2.2.2.). Treatments were compared by means of Fisher's protected LSD test.

2.3. Budding techniques

The standard budding techniques (Rousseau, 1966; Vogts & Rousseau, 1976) were refined and tested under lathe house and field conditions.

2.4. Time of grafting and budding

Grafting and budding experiments were conducted in all seasons of the year. Average success rates of experiments were compared to establish if strong seasonal influences could be discerned.

2.5. Effectiveness of grafting

The efficiency was evaluated of propagating cultivars by means of grafting as opposed to the rooting of cuttings. This was done with respect to a) the percentage success of scions taken vs. cuttings rooted, b) early survival rates of established grafted and rooted cutting plants, and c) the cost effectiveness of the two propagation methods.
a) Percentage success. An experiment comparing the percentage rooting of cuttings of the scion cultivar with percentage grafting success as well as rooting of the rootstock is presented in 2.2.3.
b) Early survival rates of established plants. In a further study the early survival rates of established grafted and cutting *Leucospermum* plants were compared. In this study unrooted cuttings of *Leucospermum patersonii* and a *L. tottum* x *L. formosum* F_1-hybrid (T75 11 02) were wedge grafted in autumn with three *L. cordifolium* selections as described 2.2.2. The three selections, "Vlam", "Mars" and T75 11 03 were also rooted from terminal cuttings as described above. Rooted transplantable rootstock cuttings with live scions, and rooted cuttings of the three scion selections, were lifted from the rooting frame at 12 weeks and transplanted directly to the field in mid-winter. Variable numbers of the plants were established at Tygerhoek Experimental Farm, Riviersonderend on a deep alluvial Table Mountain Group sandstone soil. One each of the six entries were planted together per replicate plot. The plants were mulched with black polyethylene film (1 m x 40 µm) to control weeds and improve the soil moisture status. At eight months from planting, in autumn, survival of grafted plants (both the rootstock and scion alive) and rooted cuttings were recorded and the scores were analyzed according to the methods for attribute data (Snedecor & Cochran, 1967).

3. Results and discussion

3.1. Production and handling methods of rootstocks

Rooted cuttings of both *Leucospermum* and *Protea* were found to be superior to the seedling rootstocks previously

recommended (Vogts & Rousseau, 1976). This is due to a) the ability to rapidly produce cutting rootstocks of the desired thickness (3-6 months) as opposed to seedlings (15-18 months), b) the greater uniformity obtainable with cutting rootstock material and c) the fact that clones of superior genetic constitution can be selected and produced true-to-type as rootstocks from cuttings.

Good and comparable success was attained with all three handling methods. This applies to both *Leucospermum* and *Protea* (table 1). It is thus possible to graft or bud containerised rootstocks under semi-shade in the nursery, unrooted cuttings (cutting grafting) or established plants in the field (topworking) with comparable efficiency.

Table 1 - Sample average percentages take after three months, of grafted and budded *Protea* and *Leucospermum*, using three methods of handling the rootstock.

Genus	Rooted rootstocks grafted or budded under 50% shade in nursery	Rootstock cuttings grafted or budded, then rooted (cutting grafting)	Growing plants grafted or budded on the land(topworking)
Protea	92 (graft)	92 (graft)	72 (graft)
Leucospermum	65 (graft)	93 (bud)	99 (bud)

3.2. Grafting techniques

3.2.1. Reducing desiccation of the scion

It was found that scion size could be limited to only one or two prominent axillary buds plus a small piece of their subtending leaf, approximately 0,5 cm^2 in size. This scion size effectively controls desiccation, obviating the need for a misting system. Grafting can thus be performed effectively under standard lathe house conditions with this method (figure 1).

Inclusion of the scion in a snugly fitted blue plastic (banana sleeve type) bag was the most effective method to further restrict moisture loss of the scion in *Leucospermum*.

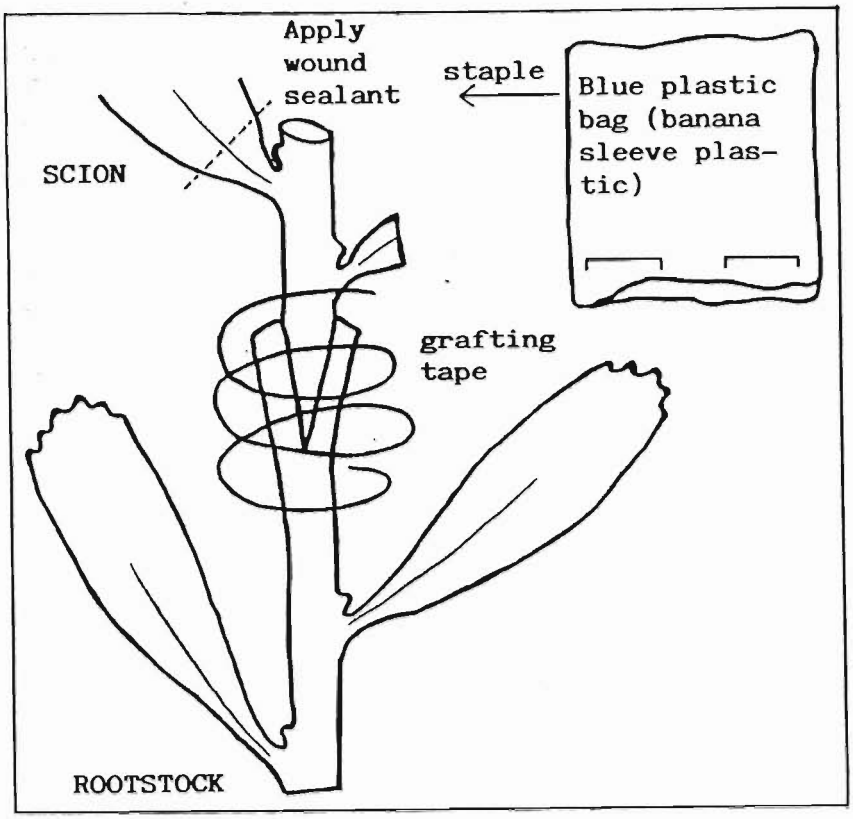

Figure 1 - Commercial wedge grafting in proteas. The use of blue plastic is not essential. Spraying with fungicide is desirable before using plastic.

This method was particularly useful for grafting *Leucospermum* under field conditions. It is however important to use disease-free scion material which should in addition be sprayed with benomyl and left to dry before covering with plastic (figure 1).

3.2.2. Securing the scion to the rootstock

The results with four methods to secure scion and rootstock and controlling scion desiccation are given in figure 2. Standard grafting with PVC-tape combined with covering the scion with a blue plastic sleeve gave the highest overall success. The 75% scions found growing on rooted cuttings after six months represent a very satisfactory success rate compared with the rooting percentage of this rootstock (figure 2) or of cuttings of most *Leucospermum* cultivars (Brits, 1986).

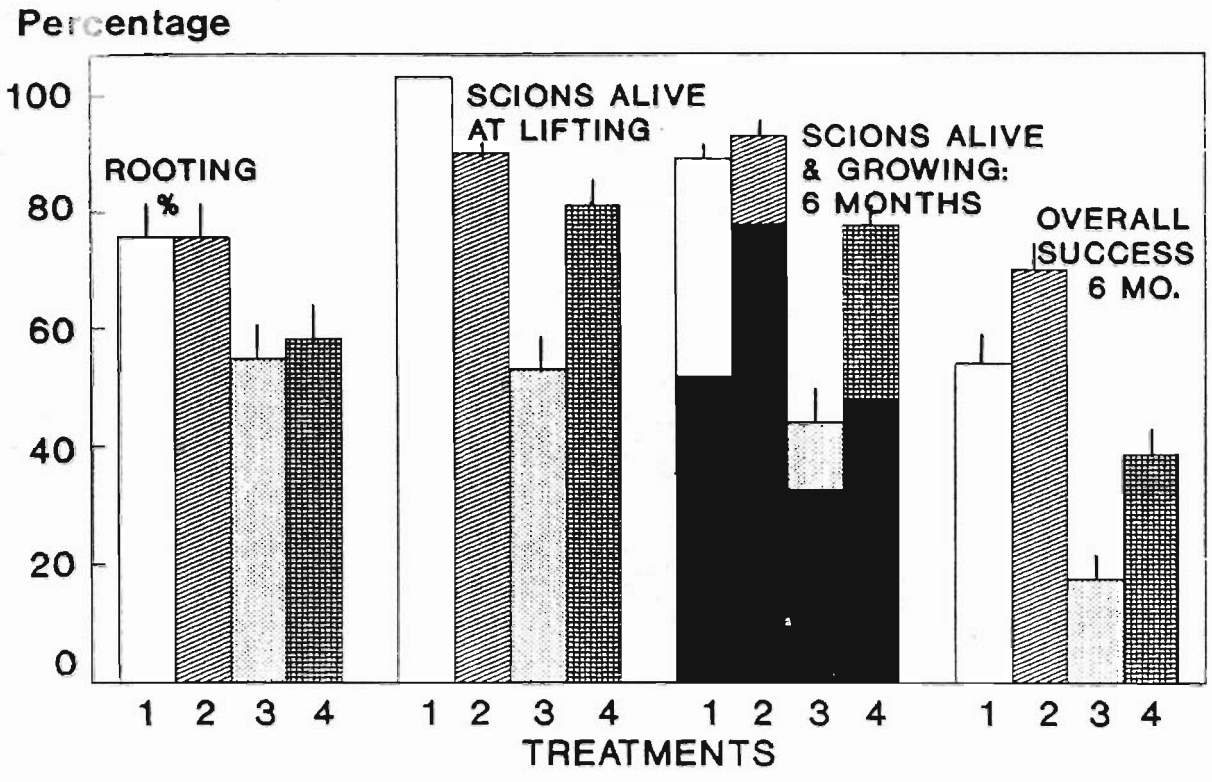

Figure 2 - Cutting grafting of 18 *L. cordifolium* clonal selections on *L. patersonii* in autumn using 4 methods to tie the scion and reduce scion desiccation: average rooting percentage of rootstock (condition of scion ignored); percentage scions alive on rooted cuttings at transplantation; cumulative percentage scions growing (■) and alive but not growing (top part of bars) on transplanted cuttings 6 months after rooting; and overall success at 6 months (scions growing on rooted transplanted cuttings of total grafted); lines on bars represent standard deviations. Treatments: 1 - wedge grafted with PVC tape; 2 - PVC tape, scion covered with blue plastic sleeve; 3 - wedge grafted with "Steri-crepe", graft union and scion covered with blue plastic; 4 - "Steri-crepe" only, crepe painted with blue PVC wound sealant after wrapping.

Although grafting with PVC tape alone (treatment 1) was also associated with both a high rooting percentage of the rootstock and high percentage of scions alive at lifting, the resumption of growth of scions at six months was significantly lower than in the case of scions covered with blue polyethylene plastic ($P<0,05$). It is not clear why blue plastic did not favour scion survival and growth when scions

were secured with Steri-crepe (treatment 3).
The use of Steri-crepe apparently suppressed rooting percentage of the rootstock as well (figure 2).

3.2.3. Uniting the scion and rootstock

In Leucospermum approach grafting of cuttings was very successful but not significantly better than wedge grafting ($P>0,5$) (figure 3). Wedge grafting is, however, more cost-effective than the latter method because of the more intensive aftercare required by approach grafting. This includes the gradual severing of the parts above and below the union, of the rootstock and scion plants respectively (Hartmann & Kester, 1983). In the case of *L. cordifolium* "Vlam" both methods gave a significantly higher percentage of scions taken on rooted rootstock cuttings compared with the rooting of cuttings of the scion cultivar (approach: $P<0,01$; wedge: $P<0,02$). This was, however, not the case with the easily rooted *L. cordifolium* "Yellow Bird".

In *Protea* approach grafting of cuttings was a total failure. The results with wedge grafting were not very satisfactory either because no significant improvement was obtained over the rooting of *Protea* scions alone ($P>0,5$ and $P=0,07$ in the case of *P. magnifica* and *P. aristata* respectively). The fact that non-grafted *Protea* rootstock cuttings rooted significantly better than scion cuttings ($P<0,01$) suggests, however, that grafting can potentially increase the efficiency of vegetative propagation in *Protea*. The results also suggest that wedge grafting could be more effective than the rooting of cuttings if the grafting technique can be improved. This relates especially to the method of securing the scion and rootstock.

The poor grafting performance of *Protea* in comparison with *Leucospermum* (figure 3) is not ascribed to incompatibility of scions and rootstocks. Low grafting percentage is rather correlated with the well-known difficulty of *Protea* to root. Furthermore the propensity of both the rootstock and scion cut surfaces to blacken excessively is probably a major factor in the low grafting success rate obtained. It is therefore necessary to develop *Protea* rootstocks which a) root easily and b) especially do not blacken excessively.

In *Protea* grafting it is also important to minimize a) the period of exposure to the air of cut surfaces i.e. of time spent to graft the scion, b) any stress on the scion, especially water stress and c) contamination. Covering the scion with a blue plastic sleeve did not give the same consistently good results found in *Leucospermum*. An approach worth investigating is the pre-treatment of *Protea* scion material.

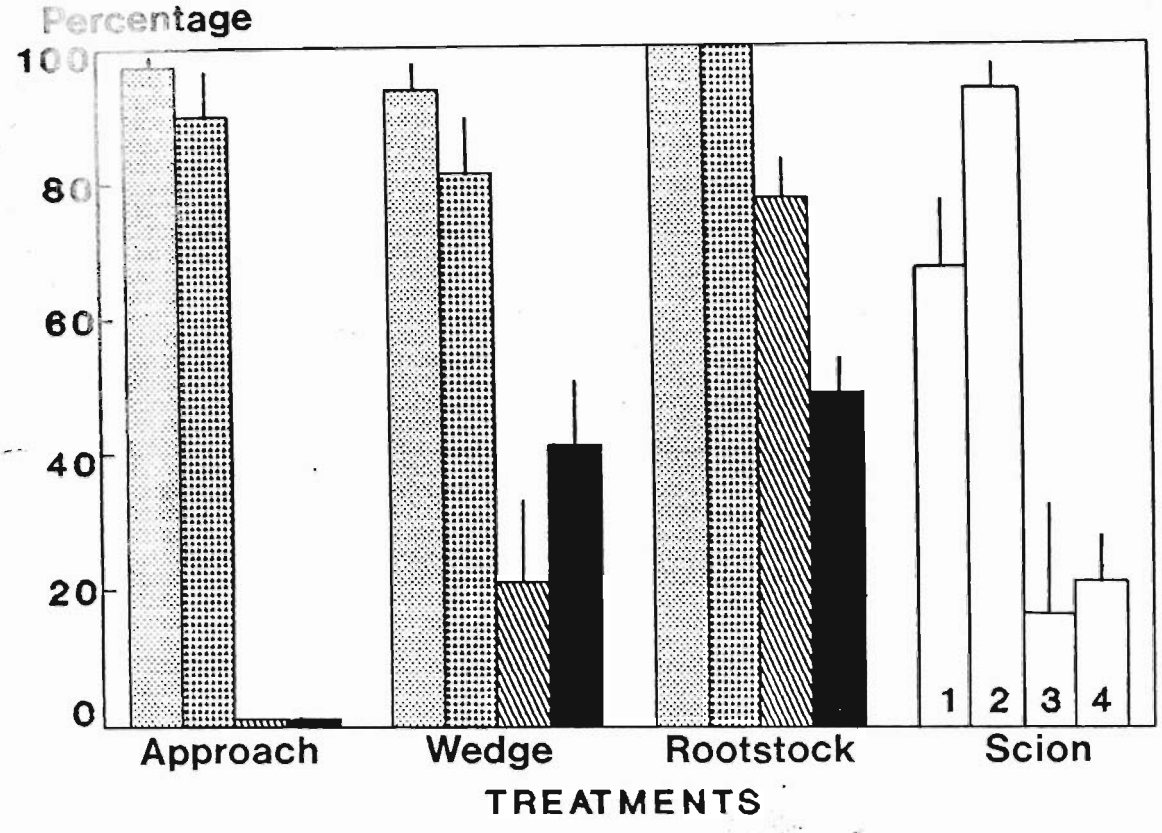

Figure 3 - Mean percentage success of combined scions taken and cuttings rooted using two cutting grafting methods, and rooting percentages of non-grafted rootstock and scion cuttings, of *Leucospermum* after 3 months and *Protea* after 5 months: Cv. Vlam (1) on *L. tottum* x *L. formosum* (▨); Cv. Yellow Bird (2) on *L. patersonii* x *L. cordifolium* (▩); *P. magnifica* (3) on *P. eximia* Rootstock 78/19 (▥); *P. aristata* (4) on *P. eximia* Rootstock 78/18 (■). Lines on bars represent standard errors.

Preliminary results indicate that cut buds of *Protea* "Andrea" (*P. magnifica* x *P. compacta*) pre-treated by washing, 12 h soaking in a weak sucrose solution and followed by a brief washing and drying until damp under clean conditions, can give a higher take when budded on *P. eximia* than cut buds rinsed only with water and budded directly. The effects of polyethylene glycol and ascorbic acid used in addition to sucrose could be investigated.

3.3. Budding techniques

A modified chip budding method was tested successfully

under both lathe house and field conditions (figure 4). Chip budding is best suited for proteas because the bark often does not "slip" readily. The budding of unrooted rootstock cuttings appears to be the most suitable method for effective large-scale commercial budding of proteas (compare cutting grafting in 2.1. and table 1). The bud should be inserted at a position approximately 2/3 up the cutting i.e. a number of leaves must be retained distally to the bud. The rootstock should be cut above the bud only after a) the cutting has rooted up to the transplantable stage; b) the bud union has healed and c) the vegetative growth season (spring) has begun.

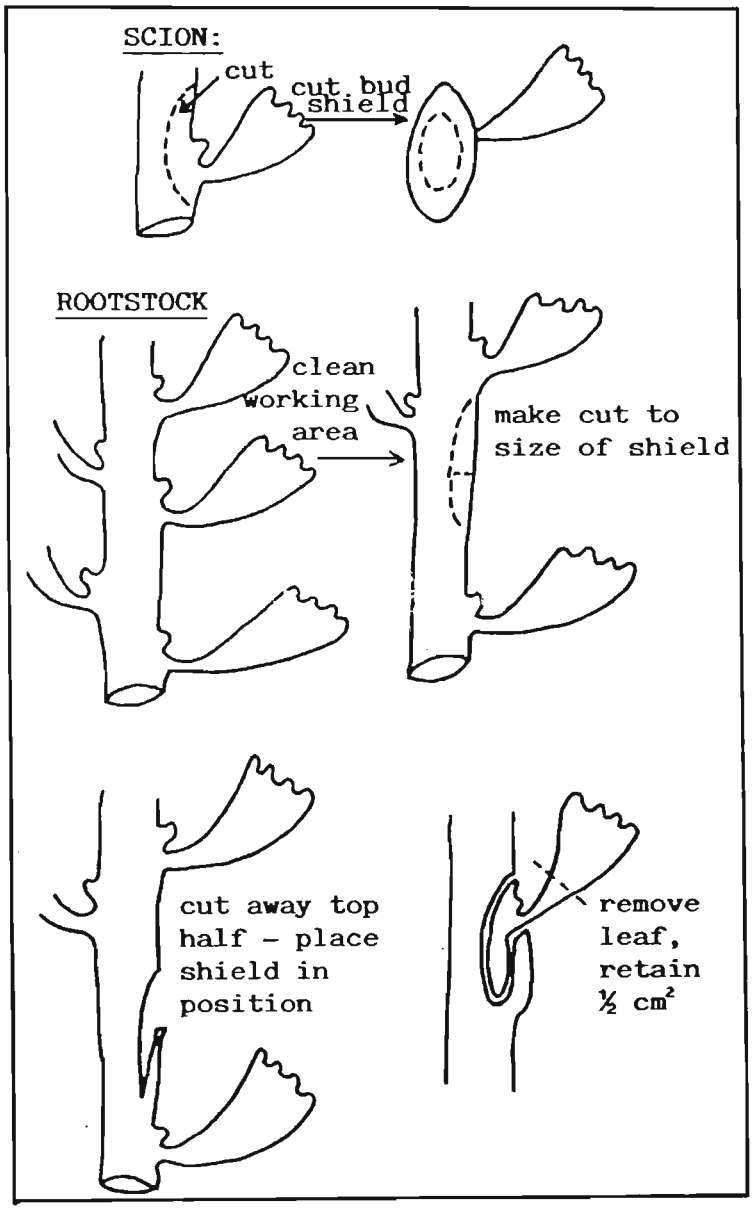

Figure 4 - Schematic representation of modified chip budding in proteas.

3.4. Time of grafting and budding

Grafting and budding can be carried out successfully from autumn to spring (table 2). Autumn to late winter is probably the best period considering the time required for wound healing prior to the resumption of shoot growth in spring.

Shoot growth, which occurs seasonally in flushes, must first resume before grafted plants can be established in the field (see for example results in 3.5 b). Scions on plants which are grafted/budded too late or have poor vigour tend to remain dormant until the following shoot growth period.

An effective schedule is, therefore, to cutting graft/bud in autumn, allow wound healing and rooting through winter followed by transplantation in containers and growth resumption in spring/summer, and establishment of strong plants in the following autumn/winter. Autumn to early winter would be best for cutting grafting considering both the healing period required and that this is also the most favourable season for rooting semi-hardwood cuttings (Jacobs & Steenkamp, 1976). Cutting grafting at an even earlier date, in late summer, will have an added benefit due to associated lower levels of disease infestation on current season's growth (Brits, 1986).

Vigorous plants (eg. *Leucospermum* interspecies F_1 hybrids) can be grafted/budded in early spring, followed by growth resumption in early summer. Alternatively vigorous plants can be cutting grafted in autumn and growing scions on rooted cuttings may be transplanted directly to the field in late spring. Special care must then be taken to minimize moisture stress in the new plants by means of additional irrigation.

Table 2 - Sample average percentage take of grafted and budded *Protea* and *Leucospermum* in autumn, winter and spring, after three months.

Genus	Autumn	Winter	Spring
Protea	-	92 (graft '77)	72 (graft '77)
Leuco-	75 (graft '77)	92 (bud '77)	99 (bud '77)
spermum	93 (bud '78)		80 (bud '77)
	92 (graft '78)		85 (graft '77)
	92 (graft '79)		98 (graft '78)

3.5. Effectiveness of grafting

a) Percentage success. A comparison of the percentage *Leucospermum* and *Protea* selections successfully wedge grafted and rooted from cuttings was made in 2.2.3. (figure 3). The results clearly indicate that difficult-to-root and disease-prone cultivars may be propagated and produced more effectively on a good rootstock than on their own root systems.

b) Early survival rates. The early survival rates of established *Leucospermum* plants were variable (table 3). "Vlam" which roots with more difficulty than the other two selections, also gave a lower survival score. Apparently proteas that root and establish with difficulty from cuttings will also propagate with relative difficulty when grafted. The low percentage successful cutting grafts of "Vlam" is ascribed mainly to the fact that grafted plants were taken to the field at an early stage before sprouting of the scions had begun. Subsequent work confirmed the importance of allowing the scion to first produce shoots of at least 5 cm long in the nursery before attempting to establish grafted plants in the field.

c) Cost effectiveness. Although the new grafting and budding methods are two or three times more costly than the rooting of cuttings, this method of propagation is nevertheless very economical relative to overall production costs. The use of grafting and budding on a commercial scale is therefore justifiable.

Table 3 - Average percentage rooted cuttings and wedge grafted rootstocks, ± standard deviation, surviving 8 months after establishment using three *Leucospermum cordifolium* selections. The same rootstock were used for grafting of the three selections.

Selection	Rooted cuttings	Cutting grafts
"Vlam"	63 ± 6	33 ± 8
T73 09 04 "Mars"	90 ± 4	92 ± 8
T75 11 03	79 ± 11	92 ± 5

4. Summary

1. Rootstocks produced from cuttings of selected clones are genetically superior to, and can be produced more effectively than seedling rootstocks.

2. *Protea* and *Leucospermum* can be grafted/budded on containerised rootstocks under semi-shade without the use of an intermittent misting system, on unrooted cuttings, or on established young plants in the field, with comparable efficiency.

3. A prerequisite for grafting is a minimal scion size consisting of two buds subtended by 0,5 cm^2 leaf sections.

4. Wedge grafting and a modified chip budding method in which the scion is secured with elastic PVC tape give the most consistent results.

5. Scions must resume growth in the nursery before grafted/budded plants can be established in the field.

6. Cutting grafting/budding should be carried out in autumn; early grafting/budding can ensure relatively low levels of disease infestation. Grafting and budding of vigorous plants using containerised or field-grown rootstocks can be done in late winter/early spring, resulting in growth resumption during the same vegetative season.

7. The efficiency of propagation by means of grafting and budding is often equivalent to or better than that obtained by the rooting of cuttings. Many of the problems associated with the root systems of proteas can therefore be overcome by commercial grafting and budding of proteas.

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ROOTSTOCK PRODUCTION RESEARCH IN *LEUCOSPERMUM* AND *PROTEA*: II. GENE SOURCES

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Abstract

Candidate *Leucospermum* and *Protea* rootstocks for cut flower cultivation were generally evaluated for vigour, rootability, scion compatibility and long term field performance when grafted. Special attention was given to adaptibility to soils with relatively high pH values and salt content. *Leucospermum* was also evaluated for tolerance to *Phytophthora* root rot. No single species of *Leucospermum* could be identified as a satisfactory rootstock and the limited available tolerance to *Phytophthora* must be exploited further. *Protea eximia, P. obtusifolia, P. mundii, P. lanceolata* and lime-tolerant ecotypes of *P. repens* are potentially useful rootstocks for most commercial *Protea* species. Interspecific hybridization in both *Leucospermum* and *Protea* holds promise to combine useful parental characteristics and utilize hybrid vigour. Successful rootstocks must be developed and tested on a regional basis.

1. Introduction

Limitations are often encountered in cultivating cut flower pincushion (*Leucospermum* R.Br.) and *Protea* L. plants on their own root systems (Brits, 1989). Similar limitations are routinely overcome in horticulture by cultivating plants on suitable rootstocks (Hartmann & Kester, 1983). Suitable rootstocks should have a series of generally useful characters eg. compatibility, vigour.

Limited but meaningful genetic variation in tolerance to *Phytophthora* root rot is apparently present within the genus *Leucospermum* (Von Broembsen & Brits, 1985). Essentially the same situation applies to tolerance to relatively high pH values and concentrations of certain salts of the soil solution, in both *Protea* and *Leucospermum*. Unlike the case with *Phytophthora* tolerance, however, the latter gene sources are better identified and reside within entire species, sub-species or ecotypes growing on substrates of known composition.

Mimetes Salisb., *Orothamnus* Pappe and *Serruria* Salisb. can be grafted successfully on *Leucospermum* (Van der Merwe, 1985). The development of succesful *Leucopermum* rootstocks

could therefore also increase the cultivation potential of these genera.

This paper reports attempts to identify and evaluate suitable rootstock types for a series of commercial *Leucospermum* and *Protea* species (table 1).

2. Materials and methods

General rootstock requirements in *Protea* and *Leucospermum* were:

- grafting compatibility;
- rooting ability for rapid production of clonal rootstocks;
- growth vigour for both high cut flower yield in grafted plants and high production on rootstock mother plants of cuttings of a suitable length, thickness and constitution (not too woody);
- mature main stem diameter suitable to accommodate thick scion stems;
- rootstock longevity >10 yrs;
- drought tolerance (<500 mm rain p.a.)
- cold tolerance (-2 to -6°C)
- tolerance of periodically wet/waterlogged conditions.

Special attention was given to nutritional stress factors:

- tolerance to pH (H_2O) values in the range 6,5 - 8,5;
- general tolerance to relatively high salt content of the soil solution and specifically high P, K, Ca and NO_3;

Table 1 - Commercial species of *Leucospermum* and *Protea* for which rootstocks were evaluated. Classification and nomenclature ex Rourke (1972; 1980; unpublished).

Genus	Taxonomic section	Species
Leucospermum R.Br.	Brevifilamentum Rourke	*L. cordifolium*
		L. lineare
		L. tottum
		L. vestitum
Protea L.	Speciosae Stapf	*P. grandiceps*
		P. magnifica
		P. neriifolia
		P. speciosa
		P. stokoei
	Ligulatae Stapf	*P. compacta*
		P. eximia
		P. obtusifolia
	Melliferae Stapf	*P. repens*
		P. aristata

Rootstocks compatible with commercial species were sought on the basis of taxonomic relationships. Comparative analyses were made of native soil types and ecological /climatical data (Rourke, 1972; 1980).

Nutritional data on selected species were collated and were correlated with tolerance factors such as ability to grow on calcareous soils (eg. Claassens, 1986). A group of 19 species within each genus which satisfied at least some of the requirements were provisionally listed and their cultivation from rooted cuttings studied. Graft and budding studies were then performed on selected groups of these species. Compatibility with *Leucospermum* and *Protea* cut flower cultivars and long term field performance of grafted plants were evaluated.

All experiments were established on acidic, Table Mountain Sandstone Group derived soils at Riviersonderend, Cape Province, South Africa unless otherwise specified. Plants were established with 1,4 m spacing across in double rows and mulched with 1m x 40 µm black polyethylene film to control weeds and improve soil moisture status. Micro-jet irrigation was applied supplementary to natural winter rainfall at 3-weekly intervals.

Tolerance to *Phytophthora* root rot in *Leucospermum*. A long-term survival study was conducted of grafted rootstocks on *Phytophthora cinnamomi* infested soil.

3. Results and discussion

3.1 Usefulness of chemical analysis of natural substrate

Claassens (1986; unpublished) found a positive relationship between natural substrate composition and the ability of *Protea* and *Leucospermum* plants to negotiate higher salt concentrations in artificial nutrient solutions. Rooted cuttings of the Durbanville ecotype of *L. conocarpodendron* ssp. viridum for example tolerated significantly higher concentrations of both NO_3 and P in pots than its close relative, the Oudebosch ecotype. The former grows on loam overlying heavy clay (table 5) and the latter on Table Mountain Sandstone Group sand overlying quartzite. Soil analysis (table 2) shows that the respective native soils differ markedly in fertility.

Claassens (1986) also compared rooted cuttings in pot experiments of *L. patersonii* with its vicariad species *L. cordifolium*, as well as the Pearly Beach ecotype of *Protea repens* with the Oudebosch ecotype. Cutting plants originating from both the former populations, which grow on calcareous soils (tables 3 and 5) tolerated significantly higher concentrations of NO_3, NH_4 and total

Table 2 - Analysis of native soils of two ecotypes of *Leucospermum conocarpodendron* ssp. viridum

Factor	Ecotype	
	Durbanville	Oudebosch
P, ppm	25	5
K, ppm	56	33
pH (H_2O)	6,35	5,5
Resistance, ohm	1214	2609

salts than their relatives originating from typical sandstone soils. These species and ecotypes of calcareous origin can in addition tolerate alkaline as well as acidic soils, in contrast with their relatives which are strongly dependent on acidic soils for normal growth (Vogts, 1980).

Claassens (unpublished results) concluded that information on the natural substrate of a species population can provide clues to its adaptibility under specific cultural conditions.

3.2 General rootstock requirements - *Protea*

Species that meet general rootstock requirements in *Protea* are given in table 3.

The genus *Protea* is slow to root (Jacobs, 1982) which suggests that prolific rooting ability in clonal *Protea* rootstocks is a very important character (Brits, 1989). The rooting ability was tested of two series of 25 randomly chosen clones of *P. eximia* and *P. repens*. In both species realised rooting percentages of clones were highly variable which concur with other results for *P. repens* (Jacobs, 1982). This suggests that rapid progress could be made by means of genotype selection. For example, *P. eximia* clone 78/19 selected for ease of rooting gave 78% rooting after three months in a separate grafting trial (Brits, 1989).

The nine most promising species (table 3) were evaluated for compatibility and field performance in grafting studies.

Grafting compatibility in *P. magnifica* grafted on *P. eximia* rootstock was, for example, tested by wedge grafting 50 two-node semi-hardwood shoot sections of *P. magnifica* on actively growing shoots of established three year-old *P. eximia* plants in spring. As a control treatment *P. eximia* was grafted on itself.

Table 3 - General characteristics of 19 *Protea* species with rootstock potential for commercial species of the sections Speciosae Stapf, Ligulatae Stapf and Melliferae Stapf*; determined from horticultural data or deduced from ecological data (ex Rourke, 1980); real or expected compatibility is based on grafting results with 9 exceptional candidates and on taxonomic relationships (Rourke, unpublished) respectively A - excellent; B - good; C - average/normal; D - unsatisfactory; relative to commercial species of the Section Speciosae eg. *P. neriifolia*

		(Horticultural data)					Tolerances (Ecological)				
Species	Real/(expected) compatibility	Rootability	Growth vigour - cutting plants	Plant size/ stem diam.	Longevity	Atypical habitat features	pH 6,5 - 8,5	Rel. high salt	Drought	Cold	Wet soils
P. aurea	B	B	A	B	D	Moist soils	C	C	D	C	B
P. burchellii	(A)	C	B	D	C	Clayey soil	B	B	D	D	C
P. compacta	A	B	B	C	D	Coastal areas	C	C	D	D	B
P. coronata	(A)	C	C	C	C	Heavy clay, moist on granite/shale	B	B	D	D	A
P. eximia (Swartberg)	A	B	B	C	B	Dry, subalpine areas	C	B	B	B	C
P. lacticolor	(B)	C	A	B	C	Moist soil, shale	C	B	D	B	B
*P. lanceolata**	C	B	B	C	C	Coast: calcareous sand/clayey gravel	A	B	B	D	C
P. laurifolia	A	D	D	B	B	Quartzite/granite /shale; dry areas	B	B	A	B	C
P. lepidocarpodendron	(A)	C	B	C	C	Sand on clay; coastal	C	B	D	D	B
P. lorifolia	(B)	D	D	C	B	Dry sand on shale	C	B	A	B	D
P. mundii	B	B	A	A	A	Moist soils	C	B	D	C	A
P. neriifolia	A	C	C	C	C	-	C	B	C	C	C
P. nitida (types)	CD	D	A	A		Granite/shale	C	B	A	B	C
P. obtusifolia	A	C	B	B	B	Coastal limestone	A	B	C	D	C
P. punctata	(B)	C	B	C	C	High altitude; dry areas	C	C	B	B	D
*P. repens**: (Pearly beach, etc.)	C	B	C	D	C	Calcareous sand (exception: shale)	A	B	C	C	C
P. roupelliae ssp. roupelliae - variants	(A)	D	C	A	A	Sandy loam on dolorite/basalt/ lime; high altitude	B	B	C	A	C
P. rubropilosa	(C)	D	D	A	A	Quartzitic; high	C	B	C	A	C
P. subvestita	(B)	C	B	B	A	Basalt/dolorite high altitude	C	B	C	A	C
P. susannae	(A)	B	B	C	C	Calcareous sand	A	B	C	D	C

Percentage take was determined at six months from grafting: *P. magnifica/P. eximia*: 73±7%; *P. eximia/P. eximia*: 59±8% (P>0,25) which indicated that the short-term compatibility of *P. magnifica* (Section Speciosae) and *P. eximia* (Section Ligulatae) is good. This is exemplary of the generally good compatibility found between members of these two sections and with *P. eximia* rootstocks in particular.

Longterm general compatibility and longevity in grafted rootstocks were studied. Containerised 18 month-old seedlings of four rootstock species were wedge grafted with variable numbers of 14 *Protea* species and hybrids covering 7 taxonomic sections. The plants were established in a non-statistical trial on an acidic, shallow, gravelly sand overlying decomposed granite under dryland conditions at Stellenbosch, Cape Province. Survival, vigour (approximating yield) and graft union quality were judged in 7-10 year-old plants over the last four years of the experiment. Vigour/yield was evaluated visually by comparing grafted plants with cutting and seedling raised plants.

The results (table 4) show that a wide range of useful compatibilities exist between species and sections, for example *P. aristata* on *P. compacta* and *P. magnifica* on *P. eximia*.

The poor survival of grafted *P. aurea* was probably due to its naturally short lifespan and poor adaptibility to dry soil conditions (table 3; Rourke, 1980). One case of delayed incompatibility was found (*P. cynaroides* on *P. aurea*) and a few cases of relatively thick scion species overgrowing their thinner rootstocks (eg. *P. magnifica* on *P. compacta*).

The species in this experiment can be divided into three groups based on rootstock requirements: those in the sections Speciosae/Ligulatae/(Exertae), Melliferae, and Protea (*P.cynaroides*). The data in tables 3 and 4 suggest that *P. eximia* and *P. obtusifolia* (Ligulatae) or their hybrids should serve well as rootstocks for the first group. *P. obtusifolia* apparently adapts normally to shallow (30-45 cm) acidic to neutral decomposed shale with a relatively high total nutrient status, on shale or clay, in the Cape as well as in California (Brits, personal observations). A highly vigorous F_1 *P. eximia* x *P. susannae* was found with superior rootability indicating that hybrids could be exceptionally useful as rootstocks. *P. lanceolata* and *P. repens* from calcareous habitats (and their hybrids) are indicated as rootstocks for *P. repens* and *P. aristata*. Species of the sections Exertae, Crateriflorae and Pinifoliae can apparently be accommodated on Exertae species, eg. *P. mundii* (table 3).

Table 4 - Field performance of four *Protea* rootstocks tested over 7-10 years under dryland conditions on an acidic gravelly Cape soil: average survival rate, vigour (yield) and graft union quality of 14 scion species and hybrids. Vigour/yield was scored visually as excellent, normal, reasonable or poor. Taxonomic sections according to J.P. Rourke (unpublished).

Rootstock		Scion				
Species (+no.of grafts)	Taxonomic Section	Species	Section	% Survival	Vigour /yield	Graft union
P. aurea (19)	Exertae	*P. aurea*	Exertae	26	Normal	Good
		P. canaliculata	Pinifoliae		Normal	Good
		P. compacta	Ligulatae		-	Good
		P. cynaroides	Protea		Poor	Poor
		P. effusa	Crateriflorae		Normal	Good
		P. grandiceps	Speciosae		Normal	Good
		P. magnifica	Speciosae		Normal	Good
		P. magnifica x *P. neriifolia*	Speciosae		Reason.	Good
P. compacta (21)	Ligulatae	*P. aristata*	Melliferae	52	Normal	Good
		P. grandiceps			Reason(Root-	
		P. magnifica			Normal	stock
		P. magnifica x *P. burchellii*	Ligulatae		Excell.	often too
		P. magnifica x *P. compacta*			-	thin)
		P. pudens	Ligulatae		Excell.	Good
P. eximia (44)	Ligulatae	*P. grandiceps*		60	Excell.	Good
		P. magnifica			Normal	Good
		P. eximia -hybrids	Ligulatae		Normal	Good
P. lanceolata (4)	Melliferae	*P. venusta*	Exertae	100	Reason.	Good
		P. sulphurea x *P. canaliculata*	Crateriflorae		Excell.	Good

3.3 General rootstock requirements - *Leucospermum*

General rootstock requirements for *Leucospermum* could be met collectively by the species listed in table 5. The six most promising candidates were tested for compatibility and performance under field conditions (table 5). *L. pluridens* was found to root poorly and *L. reflexum* to have only reasonable rootability, poor compatibility and to produce thin shoots which are difficult to graft. *L. conocarpodendron* (both subspecies) produce relatively short, thick shoots and do not root on a par with most members of the Secion Brevifilamentum (eg. *L. patersonii*

Table 5 - General characteristics of 19 *Leucospermum* species with rootstock potential for species of the section Brevifilamentum Rourke, determined from horticultural data or deduced from ecological data (ex Rourke, 1972); real or expected compatibility is based on grafting results with 6 exceptional candidates and on taxonomic relationships respectively. A - excellent; B - good; C - average/normal; D - unsatisfactory; relative to *L. cordifolium*. Rooting values (except those in parentheses) ex Jacobs, 1982.

	(Horticultural data)						Tolerances (Ecological)				
Species	Real/(expected) compatibility	Rootability	Growth vigour - cutting plants	Plant size/ stem diam.	Longevity	Atypical habitat features	pH 6,5 - 8,5	Rel. high salt	Drought	Cold	Wet soils
L. catherinae	(B)	61	C	C	C	Moist soils	C	C	D	B	A
L. conocarpodendron ssp. conocarpodendron	B	71	D	A	A	Dry slopes on heavy clay; coastal areas	B	B	B	C	C
L. conocarpodendron ssp.vir. (Durbanville)	B	(70)	D	A	A	Loam overlying heavy clay	B	B	C	C	C
L. cordifolium	A	100	C	C	C	-	C	C	C	C	C
L. cuneiforme	(C)	50	C	C	B	Dry area	C	C	B	C	C
L. erubescens	(C)	(60)	C	C	C	Dry area	C	C	B	C	C
L. formosum	B	56	B	B	B	Moist soils	C	C	D	C	B
L. fulgens	(B)	78	C	B	B	Sand over limestone; coastal	A	B	C	D	C
L. grandiflorum	(B)	66	B	B	C	Clayey dry soil	B	B	B	C	C
L. guenzii	(B)	(60)	C	B	B	Clayey moist soil	B	B	D	C	B
L. patersonii	A	(100)	A	A	A	Coastal limestone	A	B	C	D	C
L. pluridens	B	14	C	B	A	Gravelly loam; dry areas	B	B	B	B	C
L. praecox	(B)	100	B	B	B	Calcareous sand	A	B	C	C	C
L. praemorsum	(B)	56	B	A	A	Dry sand	C	C	B	B	C
L. reflexum	C	(66)	B	B	B	High moist areas	C	C	C	A	B
L. rodolentum	(C)	51	C	C	C	Calcareous sand	A	B	C	C	C
L. saxosum	(C)	(60)	D	D	B	Summer rainfall quartzitic soil	B	B	C	B	C
L. truncatum	(C)	61	D	D	C	Coastal limestone	A	B	C	D	C
L. utriculosum	(C)	29	C	C	C	Dry area; quartzitic soil	C	C	B	C	C
L. vestitum	(A)	42	C	C	C	Dry areas	C	C	B	C	C

- table 5). The latter two rootstock species appeared to have outstanding general characteristics and were tested extensively in early experiments.

The relative efficiency of rootstock cultivation of *L. cordifolium* was evaluated. Production of *Leucospermum* plants raised from seedlings, rooted cuttings and grafted rootstocks has not yet been compared. Terminal shoot sections of *L. conocarpodendron* ssp. viridum - Durbanville ecotype (table 5) were wedge grafted with *L. cordifolium* clone 75/7 and rooted; concurrently terminal cuttings of the scion clone were grafted with itself and rooted (Brits, 1989). 33 growing rooted and grafted cuttings as well as one year-old seedlings of clone 75/7 obtained from open-pollinated seed were established in spring on acidic, deep alluvial sand at Riviersonderend. The trial was planted as 11 replicate plots with one each of the three entries per replicate. Survival was recorded and vigour (yield) of the plants judged in the 5th year from planting. Although the survival rate of rootstock cultivated plants was higher than either that of scion cuttings or seedling plants (table 6) the differences were not statistically significant. Likewise average growth vigour did not differ. The results indicate that *L. cordifolium* can survive and produce normally on *L. conocarpodendron* rootstock.

The relative vigour of *Leucospermum* cultivars produced on rootstocks was compared with that of rooted cuttings on a sandy loam with relatively high total nutrient status and pH (H_2O) 5,5 in the summer rainfall region of Transvaal. Non-grafted rootstocks were included in the experiment. Groups of five plants per entry were randomized within each of five replicate blocks. The vigour of plants was judged visually after two years.

The results (table 7) show clearly that *L. cordifolium* cultivars as a group are not well adapted on this soil type compared with the three rootstocks ($P<0,01$).

Table 6 - Survival rate and average vigour (yield) of *L. cordifolium* clone 75/7 grafted on rootstock *L. conocarpodendron* ssp. viridum (Durbanville ecotype), on itself or grown from seedlings, 5 years after establishment on acidic sandy Cape soil. Vigour/yield was scored visually as good = 3, reasonable = 2, and poor = 1.

	Rootstock	Self	Seedling	P
Survival %	73	45	55	>0,25
Vigour/yield	3,0	3,0	2,8	N.S.

Table 7 - Average growth vigour of *L. cordifolium* cultivars cutting grafted on rootstocks or grown from rooted cuttings, and of non-grafted rootstocks two years after establishment under irrigation on a sandy loam in the Pretoria district, Transvaal. Vigour was scored visually: excellent = 3,5, good = 3, reasonable = 2 and poor = 1; scores were transformed to percentage values of the average score of 3,5 for non-grafted rootstock T75 11 02

Cuttings	%	Grafted	%	Rootstocks	%
Firedance	54	Firedance T75 11 02	80	T75 11 02 (*L. formosum x L. tottum*)	100
Yellow Bird	60	Yellow B. T76 01 09	66	T76 01 09 (*L. cordif. x L. patersonii*)	66
Flamespike	46	-		*L. patersonii*	80
Pink Star	49	-		-	-
Average	52		73		82

However when grafted the growth of "Firedance" improved significantly ($P<0,05$). Both grafted cultivars performed on a par with their non-grafted rootstocks. Rootstock T75 11 02 is clearly superior to T76 01 09 under these conditions ($P<0,05$) (table 7). The poor adaptibility and vigour (yield) of *L. cordifolium* on certain soils can thus be improved by means of correct rootstock choice.

3.4 Tolerance to *Phytophthora* root rot - *Leucospermum*

Leucospermum species (Von Broembsen & Brits, 1985) and clonal selections (Von Broembsen & Brits, 1989) were evaluated for tolerance to root rot caused by *Phytophthora cinnamomi* (*P.c.*). In these studies ungrafted plants raised from cuttings were grown in pots and in the field respectively.

The performance of grafted plants under field conditions of high natural *P.c.* inoculum levels has not yet been studied. A combined long term grafting and cut flower yield trial was conducted at Riviersonderend on acidic, alluvial sand with poor drainage and high natural *P.c.* levels. Two *L. cordifolium* selections and cultivars were grafted onto three rootstocks or rooted as cuttings. Each entry was replicated in 3-6 randomized blocks with 5 plants per entry. Six years after establishment survival under field conditions was recorded. The rootstocks were the previously promising *L. conocarpodendron* ssp. viridum (Durbanville ecotype) and *L. patersonii* (table 5), as well as a *L. tottum* x *L. formosum* selection (table 7).

Table 8 - Average survival of two *L. cordifolium* selections six years after establishment at Tygerhoek Experimental Farm on a soil with high *Phytophthora cinnamomi* levels, either as rooted cuttings or grafted on three rootstocks. Values[a] do not differ significantly

Selection	Cuttings/ rootstock	Survival %	P
T77 10 07	cuttings	86	
	L. conocarpodendron ssp. viridum (Durbanville)	46	<0,05
'Vlam'	cuttings	72[a]	
	L. patersonii	20	<0,05
	L. formosum x *L. tottum* (T75 11 02)	86[a]	<0,05

Table 9 - Comparative percentages mortality found in three trials, of some species, subspecies and F_1 interspecies hybrids with good general rootstock qualities: 1) pot trial artificially inoculated with *Phytophthora cinnamomi* (*P.c.*) (Von Broembsen & Brits, 1985); or grown from 2) ungrafted cuttings (Von Broembsen & Brits, 1989) and 3) grafted rootstocks (table 8) in sites with high natural *P.c.* levels and soil factors favouring disease. Casualties in 1) and 2) were positively identified for *P.c.* infection.

Species/ selection	% Mortality		
	Pots	Ungrafted	Grafted
L. conocarpodendron ssp. viridum (Durbanville)	-	62	54
L. cono. ssp. viridum - other	0	46	-
L. cono. ssp. viridum x *L. cuneiforme* (T75 11 24)	-	16	-
L. cuneiforme	40	41	-
L. formosum	0	-	-
L. formosum x *L. tottum* (T75 11 02)	-	21	14
L. patersonii	100	76	80
L. reflexum	0	33	-

The results (table 8) show significant differences in survival between a) cutting raised and grafted plants and b) rootstocks - *L. formosum* x *L. tottum* seems to be the

only promising candidate. Both clones survived comparatively well on their own root systems. Cv. Vlam has been observed to perform well under soil conditions conducive to *P.c.* root rot at Riviersonderend. *L. conocarpodendron* and *L. patersonii* which have good general rootstock qualities (table 5), failed as grafted rootstocks under field conditions. Yield of grafted plants was unaffected compared with rooted cuttings (results not presented).

The question arises if any species or clonal selections with consistently better *P.c.* tolerance can be identified from available data. Collated data of three independent trials to test tolerance of selected *Leucospermum* species, subspecies and hybrids to *P.c.* root rot show that outstandingly poor and good performers were *L. patersonii* and two hybrids respectively (table 9). T75 11 24 roots poorly (Brits, 1986) whilst T75 11 02 roots exceptionally well (Brits, 1989). *L. formosum* the one parental species of the latter hybrid, and *L. reflexum* appear to be the only species relatively tolerant to *P.c.* root rot. The data illustrates significant variability within *Leucospermum* as well as consistency in relative performance of some items.

4. Conclusions

Tables 3 and 5 show that a number of generally useful *Protea* and *Leucospermum* rootstock qualities are distributed amongst the members of these groups. However they are not combined in a single species. This implies that interspecific hybridization, backcrossing and selection within segregating progenies would have to be conducted to develop highly favourable combinations of characters. *Protea* and *Leucospermum* species hybridize comparatively easily and the hybrids show strong heterosis (Brits, 1983). The performance of selections T75 11 02 and T75 11 24 (table 9) show that the use of hybrids could be effective. Heterotic rootstocks would also be required to accommodate extremely vigorous scion cultivars eg. "Andrea" (*P. magnifica* x *P. compacta*).

The great variety of "atypical" soil types occurring within regions otherwise favourable for protea cultivation suggests that rootstocks must be developed and tested on a regional basis prior to commercial introduction. The differential reactions of rootstocks to a relatively rich soil type (table 7) supports this conclusion. Qualities required for any region must be combined if a rootstock is to succeed, eg. nematode resistance and *Phytophthora* root rot tolerance in Hawaii (Rohrbach, 1983).

A study of differential tolerance to, and chemical control of, *Phytophthora cinnammomi* in *Banksia* species and *Protea cynaroides* showed that soil saturation with metalaxyl can provide effective control of *Phytophthora* root rot (Cho,

1981). It would appear that grafted rootstocks with a reasonable degree of tolerance under field conditions, combined with chemical soil treatment, could be the most effective short term solution to the *Phytophthora* problem of pincushions.

Acknowledgements

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A TECHNIQUE TO IMPROVE THE PROPAGATION BY STEM CUTTINGS OF PROTEA OBTUSIFOLIA BUEK EX MEISN.

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Abstract

The rooting percentage of stem cuttings of *Protea obtusifolia* Buek ex Meisn., following the standard technique, is usually low. Longitudinal cuts made in the bark of the cutting base, permited to increase such percentage up to over 60%.

Introduction

South African proteas are normally propagated by seeds or by stem cuttings (Meynhardt, 1974; Jacobs and Steenkamp,1975 and 1976); Jacobs, 1983). *Protea obtusifolia* Buek ex Meisn. is a native species of the southern Cape Coast, where it grows in alkaline soils, with high values of pH (above 8), although it can be successfully cultivated in acid and alkaline soils (Vogts, 1982; Rourke, 1982). For this reason we have recommended it for the coastal areas of the north side of Tenerife, where banana is the main crop, which is actually suffering a bad crisis due to the entrance of the Canaries into the European Economic Community. The soils of these areas have a pH in the range of 6.5 or higher and an electrical conductivity in the range of 1.0-2.0 dS/m, or higher.

The *P. obtusifolia* plants, currently in the commercial plantations of proteas in Tenerife, have been grown from seeds imported from South Africa and for this reason there is a great variety amongst them. The plants differenciate in the colour and size of flower, the growth rate, adaptation to the ecological conditions, etc. There are plants with healthy and vigorous growth, whilst others grow with difficulty showing symptoms of chlorosis and other nutritional disorders. It is therefor necessary to make a selection of the most appropriate plants and propagate them vegetatively, thereby improving the existing plantations, obtaining a higher quality and uniformity for future plants.

The results obtained in our propagation unit, in the multiplication of *P. obtusifolia* by means of stem cuttings, following the standard technique (Jacobs and Steenkamp, 1975), were disappointing, the percentage of rooting was low. In order to improve on these results, bearing in mind that in certain woody species, such as juniper and azalea, wounding the base of the cuttings is favourable for rooting (Hartman and Kester, 1974), longitudinal cuts were made in the base of some cuttings, which resulted in root development at a much faster rate with a considerably higher percentage.

The purpose of the present study was to investigate the effects of these cuts in the rooting of cuttings from *P. obtusifolia*.

Material and methods

Terminal semi-hardwood cuttings, 15 cm long, were prepared from shoots of the current season's growth taken from numerous healthy and vigorous *P. obtusifolia* plants, approximately two and a half years old, grown in a commercial plantation in Los Realejos (Tenerife).

A randomized block design was employed with two treatments (A and B) and three replications. The experimental unit consisted of 20 cuttings. The total number of cuttings was 120.

In treatment A (control), cuttings prepared according to the standard technique were used. In treatment B, the same type of cuttings were employed, but in addition, four longitudinal cuts of 2 cm long, equally spaced, were made in the bark of their bases.

Cuttings were stripped of leaves on their basal half and a fresh cut was made at the base of each cutting, before dipping the basal 2 mm into a 50% ethanol solution of IBA (4 g/l) for five seconds, followed by a dip in "bencap" fungicidal powder (Brits, 1986). Then, they were planted in a mixture of polysterene foam pellets and peat moss (1:1 v/v) in plastic propagating trays, which were placed on a bed with bottom heat (25 $\pm$ 2°C) in a well ventilated greenhouse (polyethylene roof and plastic netting walls) at a 60% reduction of natural light. Mist irrigation was supplied by misting nozzles, at 50 l/h for 36 seconds, every 30 minutes between 09.00 and 17.00 h.

At 12, 16, 20 and 24 weeks from planting, cuttings were scored for adventitious root development. The following scale was used: 2 = transplantable; 1 = roots present but not transplantable; 0 = no roots (Brits, 1986). The results were subjected to analysis of variance and to the Duncan test.

Results and discussion

Nine weeks after the start of the experiment, it was observed that callus had appeared on the cuttings where longitudinal cuts had been made (treatment B), and some had roots or were starting to show them, whereas the majority of those used under the control treatment (treatment A) had no sign of callus formation. It proves therefor, that the cuts made are favourable in respect of the formation of callus and the consequent growth of roots.

As can be seen in figure 1, at 12 weeks from planting the overall percentage of rooting of cuttings with treatment B was 50%, against only 6.7% of those with treatment A. At 16 weeks the percentages were 55% and 11.7% respectively. This latter percentage, referring to cuttings with treatment A, was maintained throughout the experiment, whilst the percentage with treatment B increased to 58.3% at 20 weeks and 63.3% at 24 weeks, the duration of the experiment.

During the experiment the percentage of dead cuttings where treatment A was applied, increased from 0% at 12 weeks to 3.3% at 24 weeks, whilst the percentage with treatment B was 1.7% and 8.3% respectively. At the end of the experiment, the percentage of stem cuttings showing roots, but which were not transplantable was 3.3% with treatment A and 1.7% with treatment B.

At 24 weeks there was a significant difference in the overall percentages of rooting in treatment A and B, at a level of 1%. The technique used in treatment B, increased the rooting percentage to almost 5½ times more than that of treatment A.

Conclusions

The results obtained confirm that the propagation of *Protea obtusifolia* by stem cuttings, following the standard technique, can be improved upon, when prior to hormone treatment, 4 longitudinal cuts, 2 cm long, equally spaced, are made in the bark of the cutting base.

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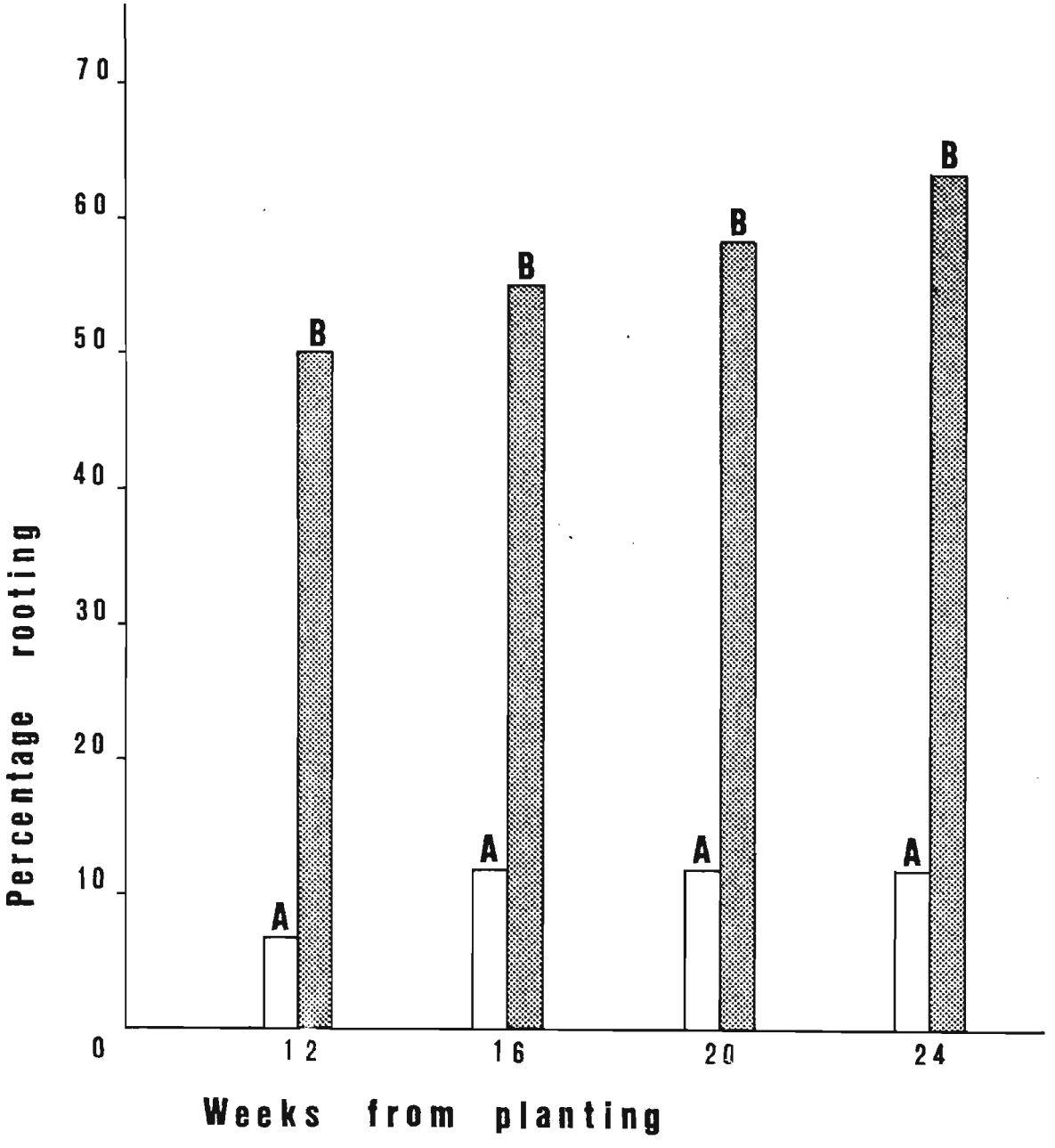
Figure 1 - Effect of basal longitudinal cuts on rooting percentage of *Protea obtusifolia* cuttings. A = control; B = cuttings with cuts.



MICROPROPAGATION OF *LEUCOSPERMUM*

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Abstract

When axillary bud explants of *Leucospermum conocarpodendron X L. cuneiforme* 'Hawaii Gold' were cultured in liquid medium of modified 1/2 MS inorganic salts, vitamins (0.25 mg/l each of pyridoxine·HCl and nicotinic acid and 0.2 mg/l thiamine·HCl), 0.2 mg/l BA and 2% sucrose, they developed proliferative bodies. When placed on filter paper bridges in shoot differentiation medium, these proliferations developed shoots which were then further elongated on agar medium. Microcuttings rooted *in vitro* were established under greenhouse conditions.

1. Introduction

Micropropagation of cut-flower protea was investigated because, in Hawaii, large quantities of vegetatively propagated plants are needed to upgrade existing fields with superior cultivars (Parvin, 1985).

Micropropagation has been successful for the following proteaceous plants: *Grevillea rosmarinifolia* (Ben-Jaacov et al., 1981), *Grevillea* hybrids (Gorst et al., 1978) and *Telopea speciosissima* (Seelye et al., 1986). Other proteas have been established successfully *in vitro* but rooting and establishment under *in vivo* conditions were not reported (Ben-Jaacov et al., 1986; Seeley et al., 1986).

2. Materials and Methods

Disinfestation procedure, initiation of culture, and culture conditions have been described previously (Kunisaki, 1989). For induction of shoot formation, proliferative bodies were placed on filter paper bridges in liquid media for shoot differentiation (Table 1) or modified 1/2 MS (Murashige et al., 1962) in which potassium and ammonium nitrates were reduced to half strength. Both media were supplemented with 0.2 mg/l benzyladenine (BA).

For root induction, microcuttings were dipped in 150 mg/l IBA solution for 10 minutes and transferred to rooting medium (Table 1) solidified with 7 g/l agar. Rooted cuttings were transplanted to potting mixture of 2 perlite:2 peat moss:1 soil and misted for high humidity.

3. Results and Discussion

Axillary bud explants developed into green, round, proliferative bodies (Figure 1) in rotating liquid medium. These bodies can be increased through monthly transfer to fresh medium. None of the other researchers successful in micropropagating proteas have reported on the development of proliferative bodies in their cultures.

Proliferative bodies did not survive when transferred from liquid to agar medium even though agar concentration was reduced to 4 g/l. However, when proliferations were transferred to filter paper bridges, they survived and developed shoots. Comparison of nutrient media of modified 1/2 MS and shoot differentiation (Table 1), both supplemented with 0.2 mg/l BA, showed that proliferations grew with greater vigor and less necrosis in shoot differentiation medium. After 3-4 months on filter paper bridges, proliferations with shoots could be transferred to agar medium of same composition. Prolonged culture on filter paper bridges resulted in more shoots becoming vitrified.

Shoots elongated when clumps of shoots were transferred to agar nutrient medium of lower BA concentration, 0.05 mg/l. In medium of shoot differentiation nutrients, basal leaves of shoots did not die as they did in modified 1/2 MS nutrients.

The procedure for rooting of microcuttings has been modified since the previous report (Kunisaki, 1989). The immersion period of cuttings in IBA solution has been reduced from 4 days to 10 minutes with the use of higher IBA concentration, 150 mg/l. Also, rooting medium (Table 1) is now being used instead of modified 1/2 MS. Comparison of media showed that roots were longer and top growth was more vigorous in our medium.

Cuttings rooted *in vitro* were established under greenhouse enviroment. Cuttings must be maintained under high humidity until roots are established in the potting medium. In an attempt to increase the survival rate of transplanted plantlets, *in vitro* rooting in other physical supports besides agar is being investigated. Of the supports being tested, perlite seems to be most promising. Unlike agar, roots in perlite develop branches and root hairs and do not break off from cuttings in the process of removal from containers. Whether greater survival can be obtained with plantlets rooted in perlite when transplanted to greenhouse enviroment is being determined.

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Table 1 - Composition of basal media for shoot differentiation and rooting.

Constituents	Medium: Shoot differentiation (mg/l)	Medium: Rooting (mg/l)
NH_4NO_3	825	619
KNO_3	475	356
KCl	150	113
KH_2PO_4	170	128
$CaCl_2 \cdot 2H_2O$	880	128
$MgSO_4 \cdot 7H_2O$	740	555
$NaH_2PO_4 \cdot H_2O$	--	128
FeNaEDTA	36.7	36.7
MS microsalts	1X conc.	1X conc.
Sucrose	20 000	20 000
Thiamine·HCl	0.4	--
Nicotinic acid	0.5	--
Pyridoxine·HCl	0.5	--

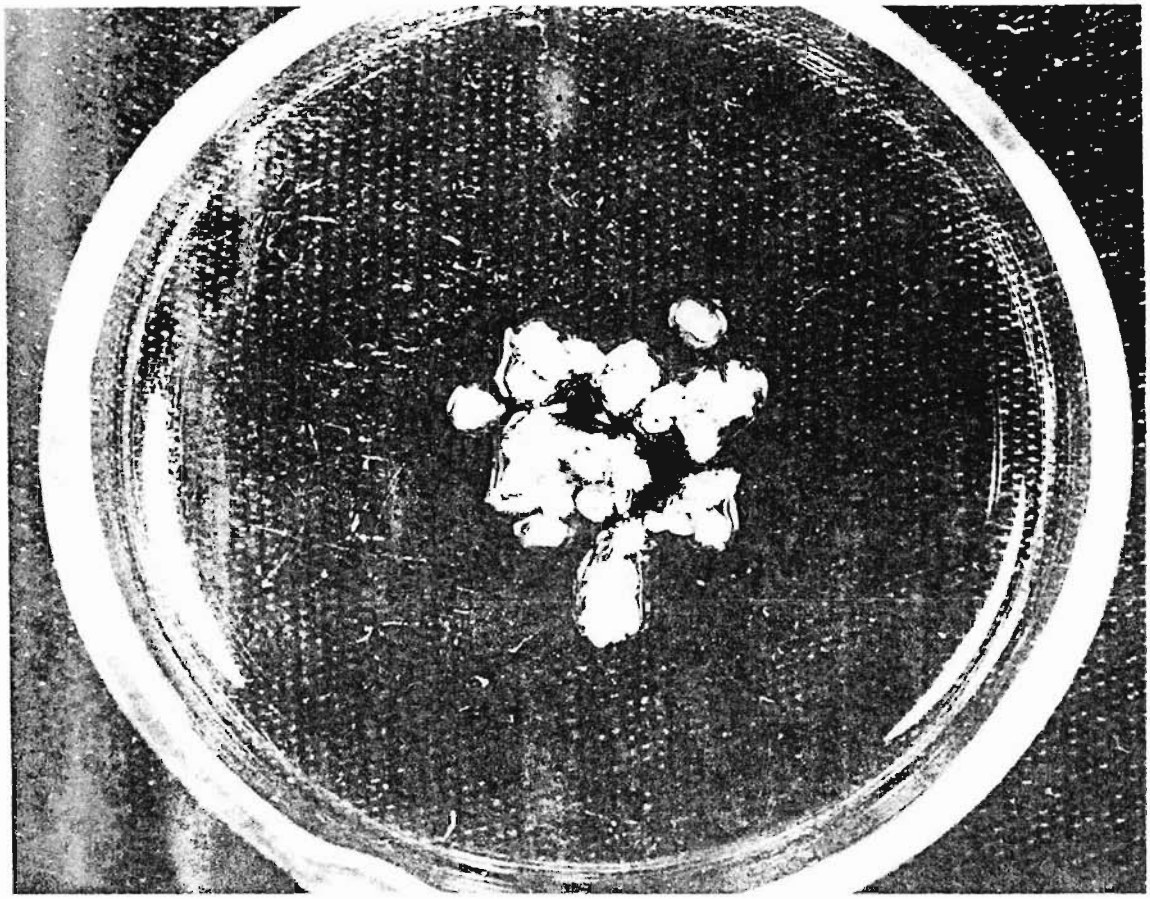

Figure 1 - Proliferative bodies initiated from axillary bud explant.

CLONAL SELECTION AND MICROPROPAGATION OF WARATAH

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ABSTRACT

Clonal selections of waratah were initiated into tissue culture. The success of initiation and establishment of cultures was dependant on the maintenance of stockplants under protected cultivation for several months prior to explanting. Adequate multiplication was achieved using a modified Murashige and Skoog (1962) medium. Adventitious roots could be initiated on microshoots *in vitro* or *in vivo*. Acclimatisation of *in vitro* rooted shoots was not achieved. Direct rooting of shoots of several genotypes was achieved using rooting powder (Seradix® N°2) and maintaining the shoots under high humidity in a fog regime (95-98%) until shoot growth recommenced.

INTRODUCTION

The N.S.W. waratah (*Telopea speciosissima*) is endemic to a limited area on the eastern seaboard of Australia. This area is under increasing pressure from urban and rural activity. Coupled with this, blooms have been harvested for sale as cut flowers, thus seed reserves have been further depleted.

Plantation cultivation is possible, but cultural problems have arisen. Waratahs are easily grown from seed but variability with respect to bloom quality and disease resistance preclude the use of seedlings for the production of a uniform, reliable product.

The University of Sydney has a large germplasm collection of waratahs taken from natural populations and from commercial growers. Vegetative propagation by cuttings presents no problems (Ellyard and Butler, 1985; Nixon, 1987). The availability of suitable material is limited however (Mullins, 1987).

Selection of superior clones of waratahs from the germplasm collection has been carried out using field trials, vase-life studies and subjective assessment based on consumer preference. Concurrently, a system for the micropropagation of waratah has been developed and has been used as a further selection criterion.

MATERIALS AND METHODS

Initiation and establishment of cultures

Vegetatively propagated stockplants of 5 selected waratah clones were maintained under protected cultivation in a growth room. Nodal segments of the terminal growth of these plants were excised and shaken in 1% sodium hypochlorite for ten minutes and rinsed three times in sterile distilled water. Single axillary nodes were transferred to a double strength iron Murashige and Skoog (1962) medium without the addition of a plant growth regulator. All cultures were incubated at 25°C ± 1°C with a 16 hour photoperiod and a photon flux density of 100 µmol m^{-2} s^{-1}. After bud burst, explants were transferred to the same medium with the addition of 1.25 µM benzyl adenine (BA) (Seelye, 1984).

Rooting and Acclimatisation

(i) Rooting *in vitro* Microshoots (15-35 mm) harvested from the fifth subculture of clone R 2/2 were placed into agar, crushed quartz or filter paper bridges *in vitro* with varying concentrations of IBA and incubated at 120 µmol m^{-2} s^{-1} until root development was observed. Rooted microshoots were transferred to a high humidity chamber (RH 90-95%) or to a fog house. The humidity was reduced over a four week period.

(ii)Direct rooting The bases of microshoots of clone R 2/2 (as above) were dipped in various concentrations of rooting powder (IBA 1, 3, 8 g kg^{-1}) (Seradix®, May and Baker, Australia), placed into a peat / perlite mix (1:2) and maintained under a fog humidity regime.

Microshoots of five selected clones were direct rooted using Seradix® powder N° 2 (3 g kg^{-1}) placed in a peat / perlite mix (1:1) and maintained under either a fog or mist humidity regime.

RESULTS

Initiation and establishment of cultures

Rates of successful initiation into culture varied between 10% (white flowering clone) and 75% (clones 63 and R 2/2). Although all clones were successfully initiated to some degree, a decline over several subcultures occurred with some clones. This could not be overcome by variations to the growth medium, growth regulator status or incubation conditions, nor was it found to be associated with microbial contamination.

Rooting and Acclimatisation

(i) Rooting *in vitro* Whilst adventitious roots could be produced *in vitro*, the plantlets did not survive acclimatisation to glasshouse conditions under any of a variety of humidity regimes trialled.

(ii)Direct rooting The optimal concentration of IBA for direct rooting of microshoots under fog was 3 g kg^{-1} (Seradix® N°2 powder) (Fig. 1). Fog was found to be superior to mist for four out of the five clones under consideration (Fig. 2). The plantlets required 6-8 weeks under high humidity before they could be transferred to glasshouse conditions.

DISCUSSION AND CONCLUSIONS

Although initiation and establishment of *in vitro* cultures of adult clones of waratah was achieved for all genotypes in this study, the multiplication rate was dependant on the genotype and the plant growth regulator status of the nutrient medium. This observation is similar to that of Seelye (1984), who found that there was a differential genotype response of adult waratah cultures *in vitro*.

Direct rooting of microshoots is becoming an increasingly popular alternative to rooting of shoots *in vitro*. The advantages over *in vitro* rooting are manifold and include a reduction in labour costs and the early acclimatisation of plantlets. In the current work, acclimatisation of shoots rooted *in vitro* was not achieved, even with careful manipulation and monitoring of plants. This may be a consequence of a variety of physiological and anatomical abnormalities, often found to be associated with plantlets produced under *in vitro* conditions (Pierek, 1988).

The technique of direct rooting of waratah microshoots developed in this study gives promise that further refinement and assessment of genotype x treatment interactions could yield a commercially applicable system of producing selected clones of waratah.

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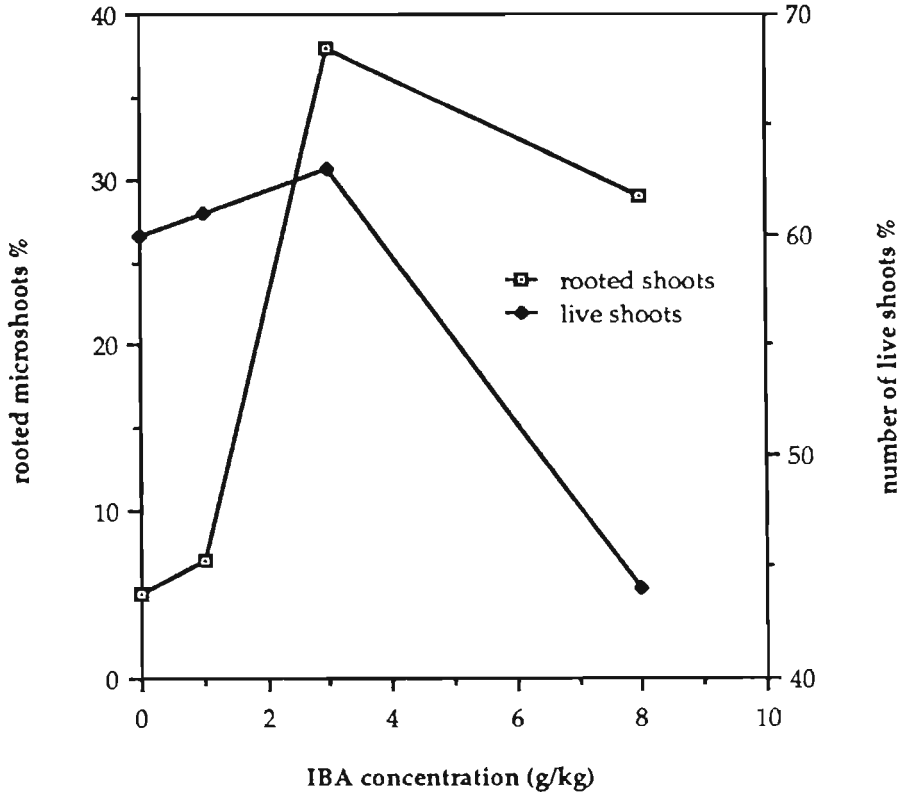

Fig. 1 The effect of the concentration of IBA (as a powder) on the number of rooted microshoots (L.S.D. 5% = 8.5) and the total number of shoots alive (L.S.D. 5% = 16.3) of clone R 2/2 after 4 weeks in fog.

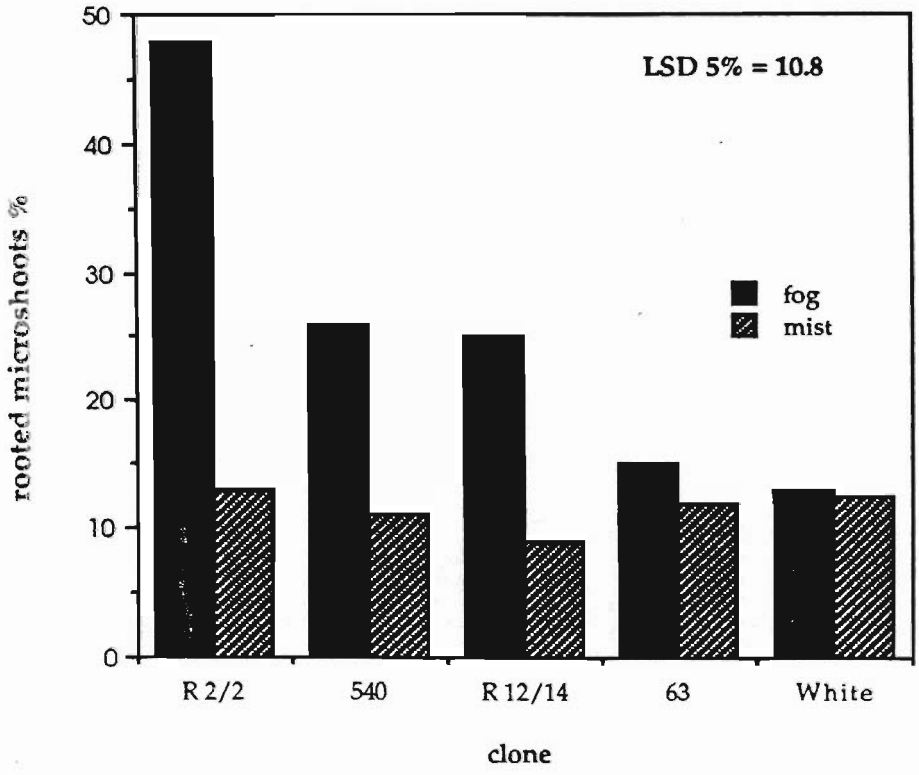

Fig. 2 Clonal waratah response to direct rooting *in vivo* under two humidity regimes .

TECHNIQUES FOR MAXIMAL SEED GERMINATION OF SIX COMMERCIAL *LEUCOSPERMUM* R.BR. SPECIES

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Abstract

Oxygen incubation, presoaking in "Promalin" (GA_4 + GA_7 + benzyladenine) and sulphuric acid scarification pre-treatment were applied as a cumulative series of treatments to freshly harvested intact *Leucospermum* achenes. Achenes were incubated under an alternating temperature regime optimal for *L.cordifolium*, which was used as a control species. The other species, originating from widely different fynbos habitats, were *L. cuneiforme*, *L. erubescens*, *L. glabrum*, *L. reflexum* and *L. vestitum*. Treatment effects on germination percentage were cumulative in most species and also resulted in similar germination rate patterns, suggesting common physiological mechanisms for germination. The promotive effect indicated for "Promalin" on *L. reflexum* and *L. erubescens* germination suggests that these species were incubated under a non-optimal temperature regime. Acid scarification combined with the other treatments gave maximal germination percentages and rates and stimulated a significant proportion of subviable achenes to germinate, as was reflected by tetrazolium viability test results. Seed coat qualities of the recently domesticated *L. cordifolium*, the "wild" species with highly viable seeds and species in which seeds were harvested prematurely (with low sinker percentages) are discussed.

1. Introduction

The germination of hybrid "seeds" (achenes) obtained in a *Leucospermum* interspecies crossing programme is a critical problem. In addition to the effects of several dormancy factors, a significant proportion of embryos in seed batches is normaly underdeveloped or aborted. This proportion can be especially high in hybrid seeds of poorly compatible parental species. It is therefore imperative to find methods for maximal germination of *Leucospermum* seeds.

Intact *Leucospermum cordifolium* (Salisb. ex Knight). Fourcade achenes require oxygenation (Van Staden & Brown, 1973; Brits, 1986a) and alternating temperatures of 8°C and 24°C for germination (Brits 1986b). Incubation of another species with nut-like fruits, *Leucadendron daphnoides* (Thunb.) Meisn., in oxygen under alternating temperatures of 10°C x 20°C leads to increased endogenous hormonal promoter levels of gibberellin and cytokinin which is followed by germination (Brown & Van Staden, 1975). Soaking *L. cordifolium* seeds in a solution of "Promalin", containing GA_4, GA_7 and benzyladenine can significantly increase the germination percentage of intact seeds Brown et al 1986). Percentage germination in *L. cordifolium* can also be strongly increased by sulphuric acid scarification (Brits & Van Niekerk, 1976). Scarification can improve the seed coat permeability to oxygen and also

relieve the constraining influence of the hard seed coat.

Low winter temperature and oxygenation by means of H_2O_2 pre-treatment act cumulatively in promoting *L. cordifolium* seed germination in seed beds (Brits & Van Niekerk, 1986). In the laboratory the combination of seed disinfection, optimal temperature regime, acid scarification, pre-soaking in Promalin, and oxygen gas incubation maximized seed germination percentage and germination rate in *L. cordifolium* as was shown in comparison with tetrazolium viability test results (Brits, unpublished data). Acid scarification, Promalin treatment and oxygen incubation, used in a factorial design, acted cumulatively in maximizing germination response.

Species of Proteaceae with nut-like fruits have similar germination mechanisms (Brits 1986b). However, they have narrow ecological amplitudes - for example *L. cordifolium* and *Serruria florida* Knight have different optimal germination temperature regimes, correlated with the general temperature regimes of their habitats (Brits, 1986b). *Leucospermum* species from different habitats could therefore be expected to have temperature requirements different from the optimal regime for *L. cordifolium*.

The species used in the present study all originate from widely different habitats. Limited resources precluded the use of all combinations in a factorial trial with six species. Consequently the hypothesis was tested that oxygen incubation, Promalin soaking and acid scarification will act cumulatively where seeds are incubated under an assumed non-optimal temperature regime.

2. Materials and methods

The species used are given in figure 1. *L. cordifolium*, of which the temperature requirements are known, was included as a control species.

The seeds of *L. cuneiforme* and *L. vestitum* were harvested from unknown sources under non-controlled conditions and donated for research purposes. The other species were grown at Tygerhoek Experimental Farm, and harvested under controlled conditions after natural release in December.

Fresh, hand sorted intact seeds of six *Leucospermum* species were scarified in (c) H_2SO_4 at 22°C for 8 minutes and washed (Brits & Van Niekerk, 1976). These as well as untreated seeds were disinfected in hot water at 50°C for 30 minutes (Benic, 1986). Seeds were then soaked for 24 h in either a 200 mg l^{-1} "Promalin" solution (Brown et al, 1986) or in distilled water. Following soaking all seeds were cleaned by rubbing off the remains of the soft, gelatinous "outer pericarp" and by washing briefly in running water (Brits & Van Niekerk, 1976). Seeds were allowed to dry off until the seed coat contained no free water and then shaken up with thiram (75% a.i.) w.p. at a concentration of 0,05g thiram powder per 100 seeds (Benic, 1986). Seeds were placed on a single layer of damp filter paper on a 10 mm sterilized sand bed within 1 ℓ flasks. Seeds were incubated either in air

or in medical grade oxygen (Van Staden & Brown, 1973) under the optimum temperature regime for *L. cordifolium* (Brits, 1986b). Control flasks (air) were covered with loose glass lids whilst oxygen receiving flasks were closed airtight with screw-on lids sealed with petroleum jelly; flasks were flushed with oxygen twice per week. The cumulative series of treatments were thus:

1) Control: incubation in air
2) Incubation in oxygen
3) Oxygen plus Promalin presoaking
4) Oxygen plus Promalin plus acid scarification

Six replications of 25 seeds per species per treatment were used in a completely randomized experimental design. Germination was recorded weekly as the number of seeds with newly emerged radicles, for 20 weeks. Germination percentages and rates were calculated, the latter by means of the formula of Heydecker (1973):

$$\text{Rate of germination} = \frac{\sum_{i=1}^{k} ni}{\sum_{i=1}^{k} Di.ni} .100$$

where k = final week of germination
Di = week of recording
ni = number of seeds germinated in week Di
i = week 1 to week k

The results were subjected to analysis of variance.

Tetrazolium seed viability tests were conducted on 6 replicates of 25 embryos per species (Brits, unpublished data).

The percentage seeds was determined of samples of 200 seeds per species sinking in water following sulphuric acid scarification and washing.

3. Results

In most of the 18 cases covering six species cumulative treatments gave step-wise increases in germination percentage (figure 1). In the three exceptions the difference between treatments were small and not statistically significant.

Germination rate gave a characteristic response pattern to treatments (figure 1). In treatments 2 and 3 the rates were slightly lower than the control treatment for most of the species. In the case of treatment 4, however, a significant increase in germination rate was found in all six species.

Analysis of variance of germination percentage gave a highly significant interaction ($P<0,001$) for treatments x species. This can be

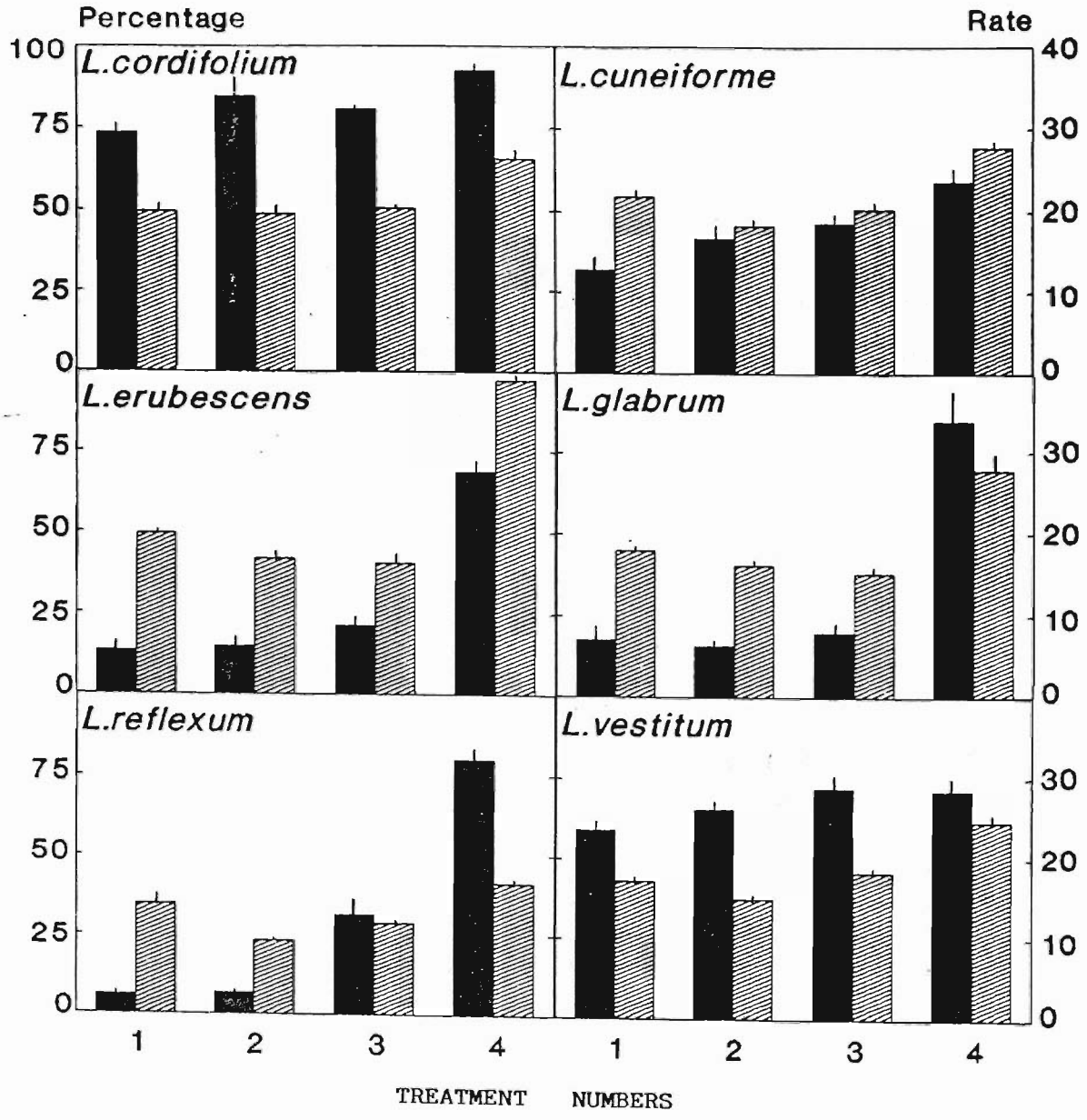

Figure 1 - Mean seed germination percentage (■) and germination rate (▨) for six *Leucospermum* species treated with four methods; lines on bars represent standard errors.

seen, for example, in the different response patterns of *L. cuneiforme* and *L. reflexum* (figure 1). Inspection of data revealed three patterns of reaction of germination percentage to treatments:

A: *L. cordifolium*. Relatively very high control value and very high maximal germination value with relatively little difference between treatments.

B: *L. cuneiforme*, *L. vestitum*. Moderately high control values and relatively low maximal values with little difference between treatments.

C: *L. erubescens, L. glabrum, L. reflexum.* Relatively very low control and oxygen treatment effects coupled with substantial reaction to Promalin and with relatively very high maximal values i.e. with very strong reaction to acid scarification.

4. Discussion

The fact that oxygen alone and in combination with Promalin tended to give a lower germination rate than controls in most cases cannot be explained. Especially since in treatment 4, in which the seed coat permeability to oxygen was strongly increased by scarification, embryos would have been subjected to a higher oxygen partial pressure than in the case of treatments 2 and 3.

The germination percentage patterns, in which germination percentage increased with cumulative treatments, as well as the similar germination rate patterns suggest that similar physiological processes are involved in the seed germination of different *Leucospermum* species. Treatment 4 thus apparently provides a maximal seed germination technique for *Leucospermum*. That maximal germination was indeed realized is supported by the close correlation between viability estimates, percentage sinker seeds and treatment 4 values (table 1).

Table 1 - Average percentage seed germination following treatment with 4 methods, for 3 *Leucospermum* groups; probability values (P) are given for comparisons of adjacent treatment means, using one-tailed t-tests. Seed viability was estimated by means of a tetrazolium test; sinker seeds denotes percentage sinking in water (see Materials and Methods).

		Treatments									
		Control		Oxygen		O_2 + Promalin		O_2 + Prom. + H_2SO_4		Viability	Sinkers
Group	Species	%	P	%	P	%	P	%			
A	*L.cordifolium*	73.6	0.08	84.7	>0.5	80.8	<0.001	92.7		94	90
B	*L.cuneiforme, L.vestitum*	45.7	0.02	53.8	0.08	59.3	0.07	65.2		56	55
C	*L.erubescens, L.glabrum, L.reflexum*	12.4	1.0	12.4	<0.001	24.2	<0.001	77.8		82	84

In freshly harvested seed batches the viability and sinker percentage of seeds are apparently closely correlated (table 1).

The high treatments x species interaction can possibly be explained by variable seed coat permeabilities to oxygen in different groups (table 1):

A: In *L. cordifolium* the seed coat would have been fully developed as was indicated by both the high viability and percentage sinker estimates as well as by the maximal germination obtained of almost 93% (table 1). The present *L. cordifolium* population is semi-domesticated i.e. it has been artificially raised from seed for more than 10 generations. Since seed dormancy usually is genetically variable (Roberts, 1972) artificial selection for a more permeable seed coat could have occurred. This could explain the high germination percentage in control seeds. A high control germination percentage for this seed source, harvested in a different year, was also found in an earlier experiment (Brits, unpublished data).

In addition incubation of *L. cordifolium* seeds under its optimum germination temperature regime would have contributed to a relatively high control value.

B: *L. cuneiforme*, *L. vestitum*. A similar response pattern as was found in *L. cordifolium*, but at a lower level (table 1) likewise suggests relatively permeable seed coats for these commercially harvested species. The low average viability and especially low percentage sinker estimates suggest that the seeds of these two species were prematurely harvested. This conclusion is supported by the relatively low maximal germination value obtained. Premature commercial harvesting of *Leucospermum* seed is a common practice. Prematurely harvested seeds can result in incompletely developed, more oxygen permeable seed coats which determines both a relatively high control value and a poor response to oxygenation (Brits & Van Niekerk, 1986). The latter reactions are also given by floater seeds (with poorly developed embryos) as opposed to sinker seeds (Van Staden & Brown, 1973).

C: *L. erubescens*, *L. glabrum*, *L. reflexum*: seeds of these species, harvested under controlled conditions, gave a high average maximal germination percentage, viability estimate and percentage sinkers, as in *L. cordifolium* (table 1). The very low control germination percentage however suggests that either a non-optimal temperature regime could have contributed to low control values, or that seed coats were much less permeable to oxygen than in *L. cordifolium*. Surprisingly incubation in oxygen alone (treatment 2) did not increase germination percentage. Oxygen could not have been inhibitory to these seeds as was suggested for *Leucadendron tinctum* Williams (Brown & Dix, 1985) since treatment 4, in which presumably the highest oxygen partial pressure was realised, gave maximal germination values approaching viability estimates. The results therefore suggest that non-domesticated *Leucospermum* species have highly impermeable seed coats in intact mature seeds.

5. Conclusions

The following roles are proposed for each in a series of cumulative treatments required to maximize germination percentage in *Leucospermum:*

1) Oxygen incubation increases the oxygen partial pressure required for germination of seeds with intact seed coats; oxygen incubation enhances germination also in acid scarified seeds, as was found in *L. cordifolium* (Brits, unpublished data). Thus scarification cannot substitute for oxygen incubation in realizing maximal germination response.

2) Promalin pre-soaking provides essential hormone(s) which act as promoters of germination. Thus the level(s) of endogenous hormones are increased/complemented by applied hormones, especially where sub-optimal environmental factors prevent synthesis/interconversion of endogenous hormones. This may be the case where seeds are incubated at non-optimal low and high temperatures.

In *L. cordifolium*, which was incubated under an optimal alternating temperature regime, Promalin treatment gave no significant response (table 1). *L. reflexum* in nature, however, germinates at substantially higher altitudes than *L. cordifolium* (Rourke, 1972) i.e. at lower environmental temperature. Its seeds would therefore require a relatively cool germination temperature regime (Brits, 1986b). *L. erubescens* on the other hand, grows on hot, dry, north facing slopes (Rourke, 1972) i.e. it germinates at higher average temperature in nature than *L. cordifolium*. Both *L. reflexum* and *L. erubescens* gave substantially incrased germination percentage with added Promalin compared to oxygen incubation alone.

3) Scarification augments germination by a) increasing testa permeability to oxygen, especially in the case of wild species and b) reducing mechanical constraint of the hard seed coat, thereby facilitating germination of embryos with a low viability (Brits, unpublished data).

Dormancy factors (e.g. high temperature requirement) are apparently variable in their expression in seed populations. For example, a seed population does not require one threshold low temperature level or duration of temperature for the breaking of low temperature dormancy of all seeds (Brits & Van Niekerk, 1986). Rather some moistened seeds will germinate even under constant high temperature, with increasing numbers responding to progressively lower temperature (Brits & Van Niekerk, 1986; Brits, 1986b). This could explain why the progressive fulfilment of environmental requirements give step-wise increases in germination percentage.

Acknowledgement

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FLOWER DEVELOPMENT IN *LEUCOSPERMUM CORDIFOLIUM* 'VLAM'

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Abstract

Flower buds of *Leucospermum cordifolium* 'Vlam' were sampled at 2 week intervals from 1 October 1988 through 9 January 1989, and the development of florets was studied in paraffin sections. For the period of flower head development, heat units were accumulated from a base of 6°C, and solar radiation was also accumulated. At a bud size of 7 mm, only involucral bracts were found, but floret development began in mid-October as the bud length reached 10 mm. Floret development proceeded slowly through December and more rapidly in January until flower anthesis was attained in February. Approximately 1/4 of the heat units and 1/3 of the solar radiation units were accumulated during the last month of the 3-1/2 month development period.

1. Introduction

Flowering in *Leucospermum* has been studied with the goals of being able to predict and control it for commercial flower producers. To this end, a model utilizing heat unit accumulation was developed using the date of primary flower removal as a starting point for the development of a secondary inflorescence (Jacobs and Honeyborne, 1979). A linear relationship between mean daily temperature and rate of development was found, and there was little variation in the heat unit accumulation to the maturation point of 90% of the flowers. Secondary flowers of the cv. Golden Star required about 925 heat units above a 5.8°C base temperature to mature 90% of the flowers, but fewer days were required when primary inflorescence removal occurred in late spring than in early spring. This could be attributed to a greater heat unit sum per day, @ 8.5 early on to @ 20 by mid-summer.

Light intensity has also been implicated in the control of flowering in *Leucospermum*. While the reduced quality response to low light intensities is similar to that of other flower crops (Jacobs and Minnar, 1980), a quantitative response has been proposed (Jacobs, 1983). Heavy shading during summer prevented flower initiation while factors leading to flower initiation were suggested to be prevalent at high light intensities. Flowers were initiated only on shoots which had stopped elongation growth, a factor brought on by stress, unfavorable shoot/root ratio, or plant maturation (Jacobs, 1985). Napier (1985) found that shading plants at the onset of the induced state led to reduced carbohydrate in the leaves and a loss of the induced state.

After shoot growth stops, the upper axillary buds enlarge, producing bracts without floret initials. These bracts make up the involucre bracts which cover the peduncle. Floret initials begin to develop under the influence of short daylengths and high light intensities at about the same time each year (Jacobs, Napier, and Malan, 1986; Malan and Jacobs, 1987). Night interruption with a light intensity of at least 3.7 uE prevented induction (Malan and Jacobs, 1987).

Floret differentiation is normally completed by early winter, and a rapid enlargement phase follows leading to anthesis in late winter or early spring.

According to Jacobs (1980), the change from vegetative to reproductive growth takes places during a relatively short period for long shoots. Deheaded plants lose their capacity to re-initiate an inflorescence during winter, a condition which may be more related to light energy relationships than photoperiod, but little inflorescence development occurred on these shoots if deheaded as days were lengthening in summer.

Differences in response to shading, shoot length, and deheading have been shown between *L. cordifolium* x *L. tottum* 'Golden Star' and *L. cordifolium* x *L. lineare* 'Red Sunset' (Jacobs, 1983; 1985).

In South Africa flowering occurs chiefly between September and early November while in California, the flowering period for *Leucospermum* is offset by 6 months. In Hawaii, *Leucospermum* selections begin to flower in late January and continue into March or early April. South African growers desire to delay flowering to bring a crop in during the winter holiday season of the northern hemisphere while in Hawaii, growers would like to advance flowering for the same reason. Deheading has been successful in delaying flowering in South Africa (Brits, 1986; Jacobs and Honey-borne, 1978), but an understanding of flower induction, initiation, and development is still necessary to determine if advanced flowering can be achieved.

These studies were undertaken with the cv. Vlam because it is now a widely grown selection of *Leucospermum* with desirable red flowers. The objective of this work is to provide baseline values for heat unit and solar unit accumulations and the timeframe in which these act during the development of the inflorescence to anthesis.

2. Materials and Methods

Ten enlarging buds were collected biweekly from 7 year old plants of *Leucospermum* cv. Vlam growing in open fields at the Maui Agricultural Research Station, Kula, Hawaii, and preserved in a formalin-aceto-alcohol solution (Johansen, 1940). Following measurement of their lengths and diameters, the buds were prepared for sectioning and staining (tannic acid-ferric chloride and safranin) to examine the extent of floret initiation and development. The first buds were sampled October 1, 1988, when they were about 7 x 5 mm in size. The last buds were collected January 9, 1989 and measured about 25 x 15 mm, including the elongating stalk. Field measurements continued until about 50% of the flower buds present had begun anthesis, approximately mid-February.

Maximum and minimum temperatures and solar radiance (MJ/day) were recorded by a Campbell Scientific Micrologger (Model CR-21). The daily mean temperature (T_{mean}) was calculated as $(T_{max} + T_{min})/2$. A base temperature of 6 C was used for heat unit accumulations over a time span beginning with 1 September 1988: $SUM\ T_{1\ to\ n} = (T_{mean} - 6)_{day\ 1} + \ldots (T_{mean} - 6)_{day\ n}$. The summation periods corresponded to the sampling dates.

3. Results

3.1. Environmental conditions

Table 1 shows the average values by month for maximum, minimum, and mean daily air temperature and solar integral. The weather conditions were considered normal for the site, neither exceptionally cold nor warm through January, 1989. During February, two periods of cloudy weather reduced the solar integral average.

Table 1. Monthly mean values for daily maximum, minimum, and mean air temperatures, daily solar integral, and photoperiod at the Maui Agricultural Research Station, Kula, Maui, Hawaii.

Month	Sep	Oct	Nov	Dec	Jan	Feb
Mean max ^{o}C	25.0	24.4	23.3	21.5	21.1	20.6
Mean min ^{o}C	15.3	15.4	15.0	13.8	13.1	13.4
Daily mean ^{o}C	19.4	19.0	18.4	16.9	16.3	16.2
Solar integral (MJ)	17.1	14.5	12.5	11.7	13.6	11.8
Sunshine duration (min)						
Longest	753	717	682	658	673	703
Shortest	719	683	658	652	653	673

[z]Calculated as the average of hourly temperature readings.

Heat unit accumulation (base temperature = 6^{o}C) at each bud sampling date showed a gradual decrease from about 14 units/day in the fall months to about 10 units during December. The total number of heat units accumulated from 1 September until 50% flowering in February was 2408.

The daily solar integral showed a similar decrease, ranging from a maximum of 26 MJ/day in September to about 7 MJ/day during cloudy periods in December and February. Approximately 2200 MJ were accumulated from 1 September to 50% flowering in mid-February. Accumulated heat and solar units showed a relatively straight line increase during the observation period (Figure 1). Had the daily mean temperature been calculated from hourly readings instead of the max-min average, the degree day sum would have been about 400 units less, an important factor to keep in mind if attempts to repeat these observations are made.

3.2. Flower bud development

The rate of flower bud length increase was gradual until mid-December. Bud diameter tended to increase gradually until 12 - 15 mm was reached, which was about the same time as the flower stalk began to elongate. During January, the flower stalk elongated to about 1/3 the overall inflorescence length, reaching 1/2 in mid-February when 50% bloom was determined (Figure 2). Diameter increase

was slow until January when it doubled and doubled again just before bud opening (Figure 2).

While the first buds sampled October 1 showed prominent bract development, floret primordia had not begun to develop and the apex diameter was less than 100 microns (Figure 3A). The first meristematic activity leading to floral primordia appeared in mid-October and the apex had broadened to 130 microns diameter (Figure 3B, 3C). By mid-November, 3 or 4 cycles of florets had developed (Figure 3D), and by late November the perianth and stamens were initiated (Figure 3E) and stylar elements were detected on the most advanced florets (Figure 3F). Stamen and pistil-late elongation proceeded through December and January Figure 3G, 3H), with one stamen adnate to the perianth and three free. There were 5 to 7 cycles of florets developed by the time buds reached a length of 15 mm by 12 mm wide and 8 to 10 cycles of 2 spirals on a 25 mm bud in mid January (Figure 4).

During flower stalk lengthening in late December, there seemed to be a slowing in the development of organ primordia on recent florets. However, the sample was small, and the large buds were difficult to section. The relationship between floret size and flower bud length is shown in Figure 5.

3.3. Relationships between development and temperature and heat unit accumulation

Plots of bud length and width were made against heat unit accumulation and solar unit accumulation (Figures 6 and 7). The sigmoid nature of the growth curve is evident to the peak where harvesting would occur. A plot of log-length flattened the curves and a regression of log-length on heat and solar unit accumulation showed a nearly linear relationship (Figures 6 and 7). Totals of 1536 heat units and 1162 solar units were accumulated from floret initiation until 50% bloom was achieved. Twenty-five percent of the heat units and 32 percent of the solar units were accumulated during the last month of development.

4. Discussion

Comparison with Jacobs and Honeyborne's 925 heat units (base = 5.89 C) to mature 90% of flowers of 'Golden Star' shows 'Vlam' to be a slower cultivar, by about 3 weeks or an additional 240 heat units. It is probably more like 'Red Sunset,' which requires a month more than 'Golden Star' in terms of its development. Since the 'Vlam' flower bud was already initiated October 1 but floret primordia were not visibly active until almost 3 weeks later, an estimate of about 120 days for flower development also compares well with the development period of about 100 days for Jacobs and Honeyborne's (1978, 1979) secondary flower heads of 'Golden Star' when stimulated into development by removal of the primary inflorescence in mid-winter.

The average daily heat unit accumulation for 'Golden Star' ranged from about 8.4 with early deheading to 11.1 with late deheading. During the developmental period of 'Vlam' about 12.8 heat units/day were recorded from the beginning of floret initiation to 50 percent flowering in the field. In South Africa, the attempt to delay flowering benefits from the higher temperature and light levels after the deheading period as development is even faster the later deheading takes place. It could be difficult to bring flowering forward in Hawaii,

especially if a greater heat unit accumulation would be necessary.

Given the assumption that flower initiation in *Leucospermum* occurs in response to high light intensities during short days, it would seem that growing areas which provide high solar integrals during the short photoperiod portion of the year may be favored with more rapid development if temperature is not limiting. It would be desirable to compare development of flower buds of this cultivar in several different locations to determine the relative influences of the solar integral and temperature. The marked sensitivity of both initiation and development of *Leucospermum* inflorescences to reduced light intensity suggests that attention be given to this factor in attempting to modify the season of flowering.

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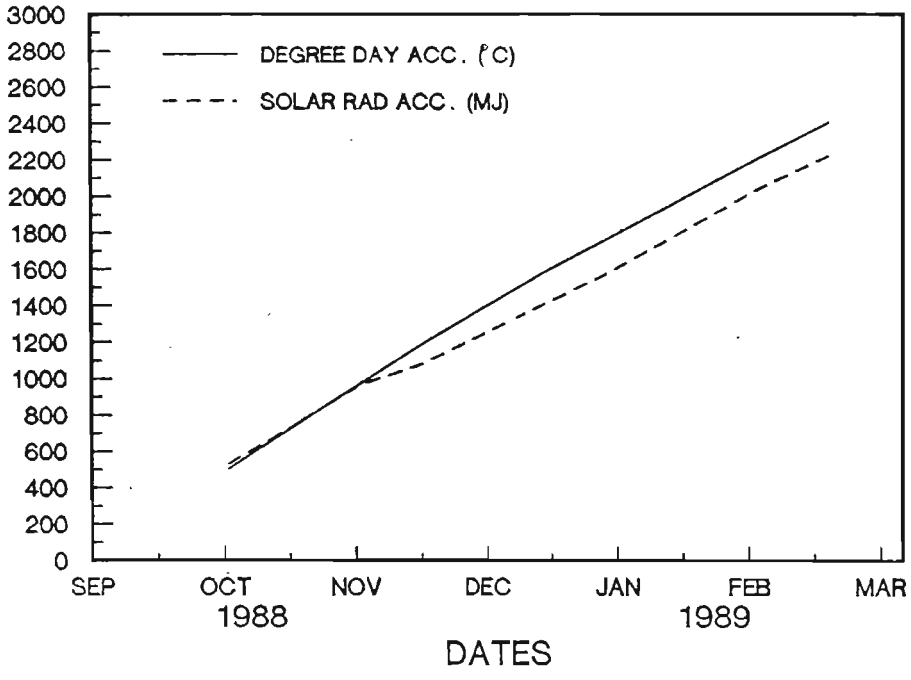


Figure 1. Accumulation of heat units (^{o}C) above a base of 6^{o}C and of daily solar radiation (MJ) from September 1, 1988, through February 15, 1989 at Kula (Maui), Hawaii. October 1 represents the first sample date and February 15 the date for which an estimated 50% harvest was attained.

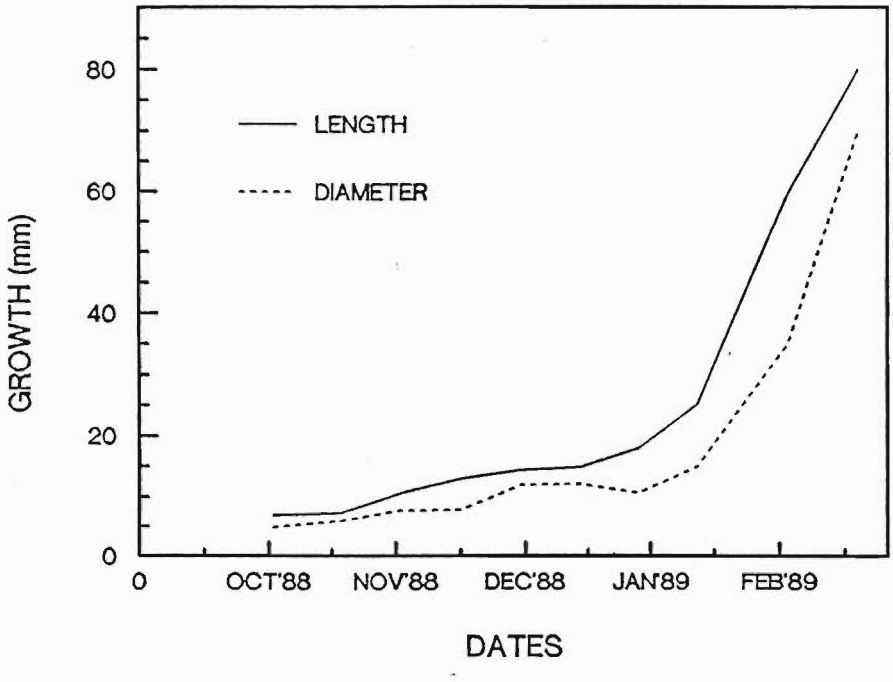


Figure 2. Dimensions of the flower bud of *Leucospermum cordifolium* 'Vlam' from the first sample, October 1, 1988 to Feburary 15, 1989 (a ready-to-flower bud). Each point is a mean of 10 buds.

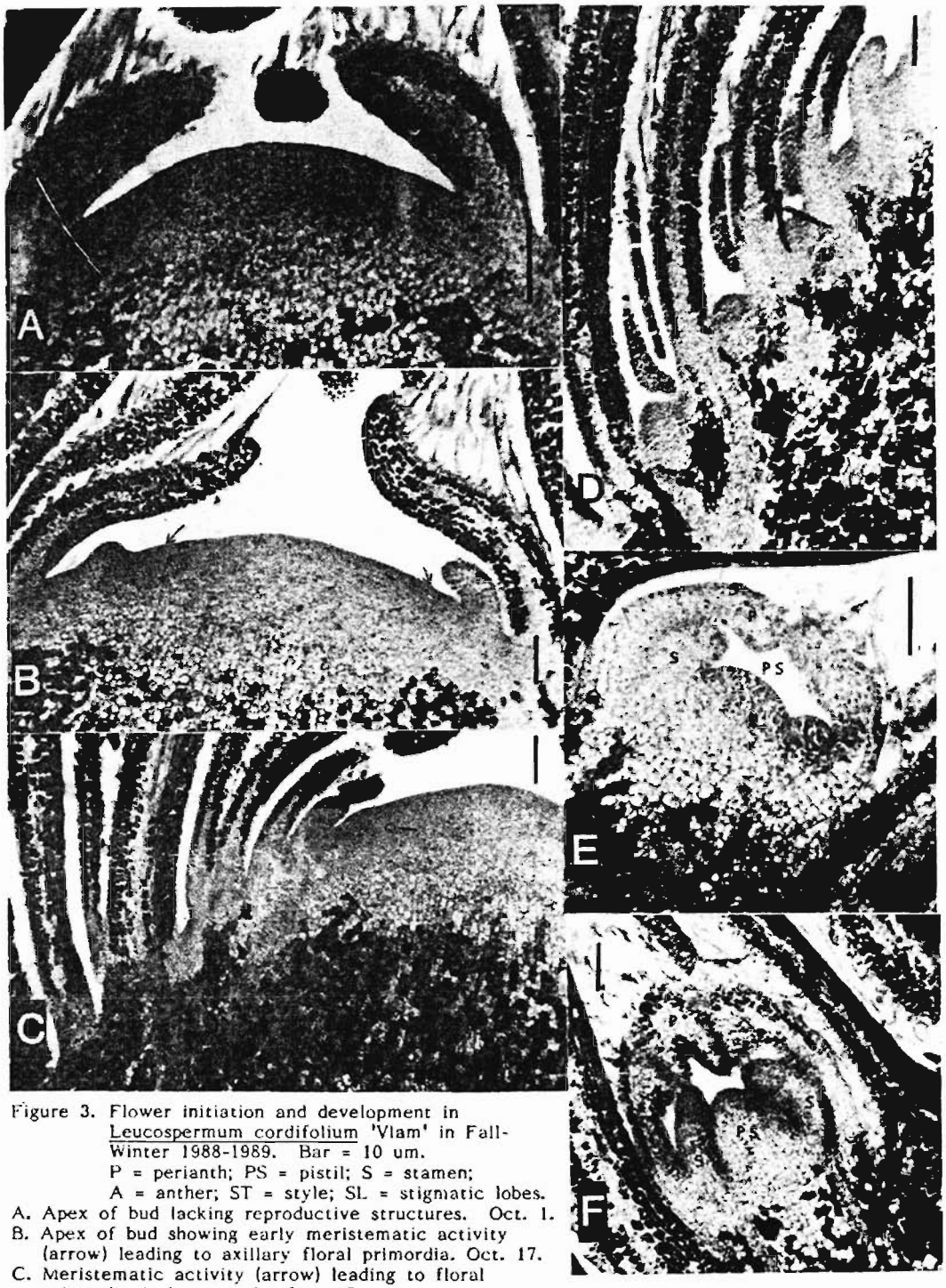
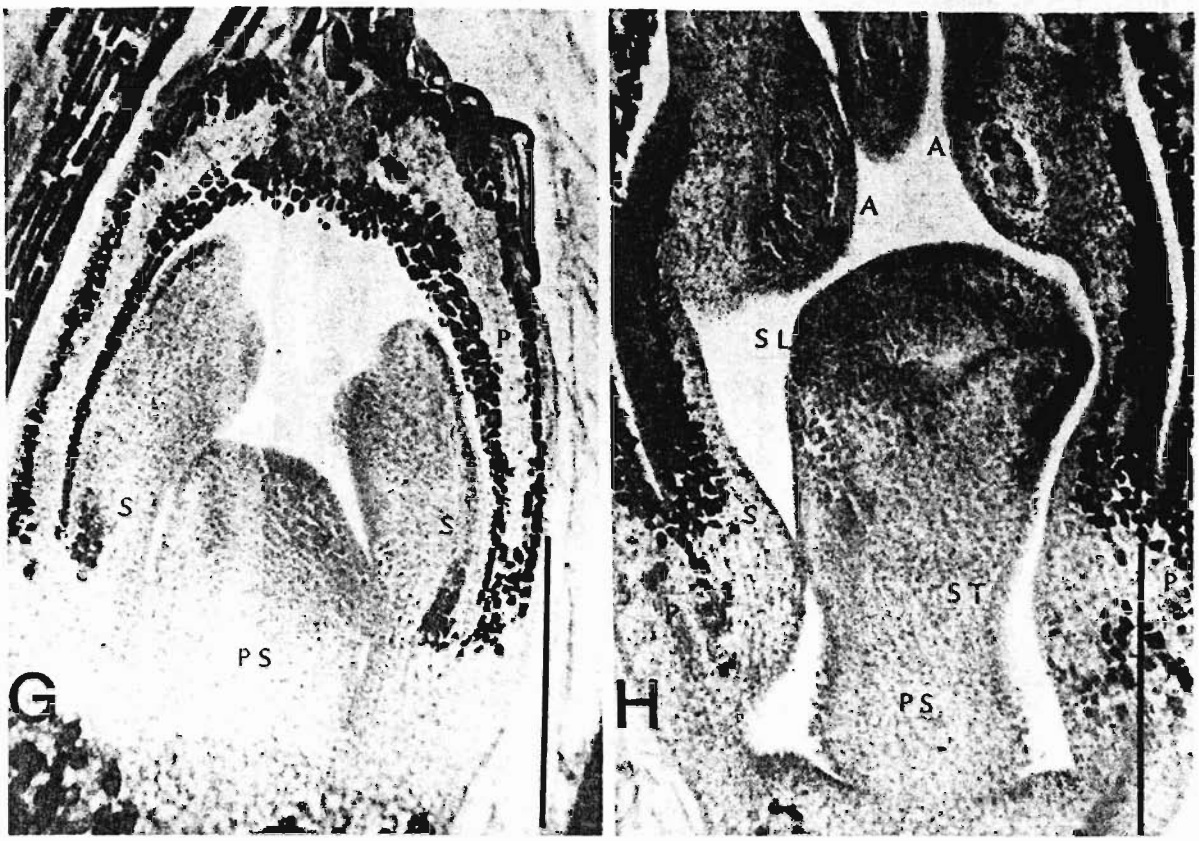


Figure 3. Flower initiation and development in Leucospermum cordifolium 'Vlam' in Fall-Winter 1988-1989. Bar = 10 um.
P = perianth; PS = pistil; S = stamen;
A = anther; ST = style; SL = stigmatic lobes.

A. Apex of bud lacking reproductive structures. Oct. 1.
B. Apex of bud showing early meristematic activity (arrow) leading to axillary floral primordia. Oct. 17.
C. Meristematic activity (arrow) leading to floral primordia in bract axils. Oct. 17.
D. Series of axillary floral primordia. Nov. 14.
E. Floral primordium with perianth, stamen, and pistillate development.
F. Floral primordium with perianth, stamen and pistillate development; about 2 weeks older than E.

Figure 3 (continued)



G. Early stages of anther and pistil development. Bar = 50 um.

H. Anther and pistil development at 4 - 5 weeks before floret opening. Bar = 50 um.

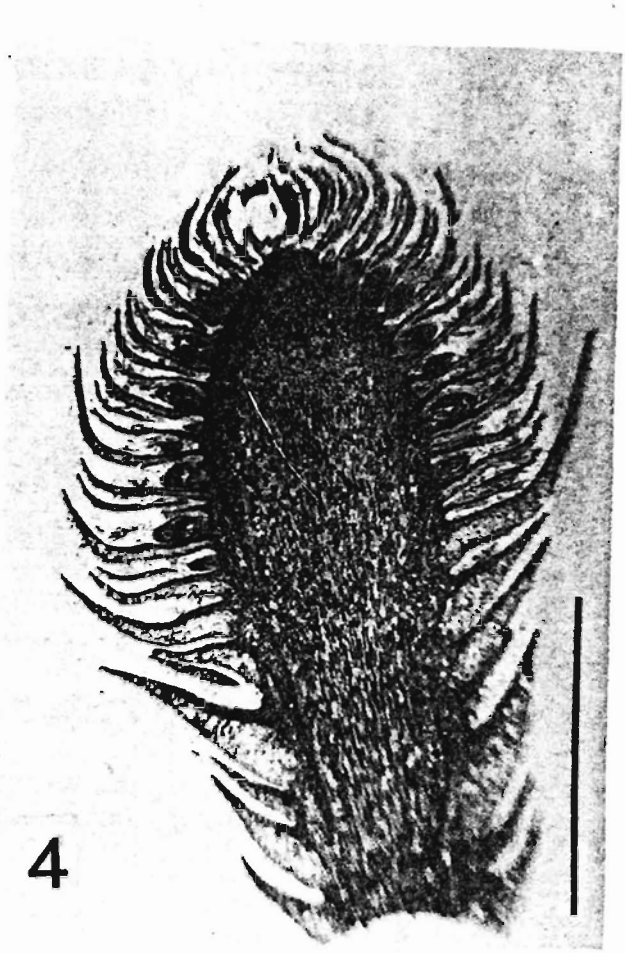


Figure 4. Inflorescence 4 - 5 weeks before first florets would open. January 9. Bar = 10 mm.

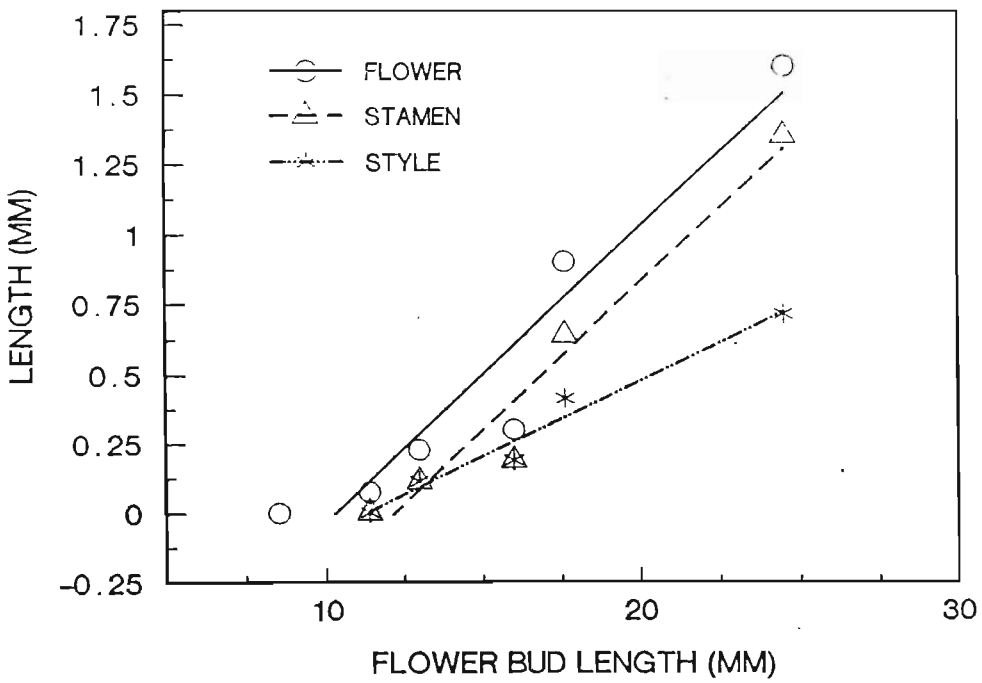


Figure 5. Dimensions of floret, stamen, and style length versus flower bud length from primordium development until 1 month before flowering. The timeframe represented is approximately 3 months.

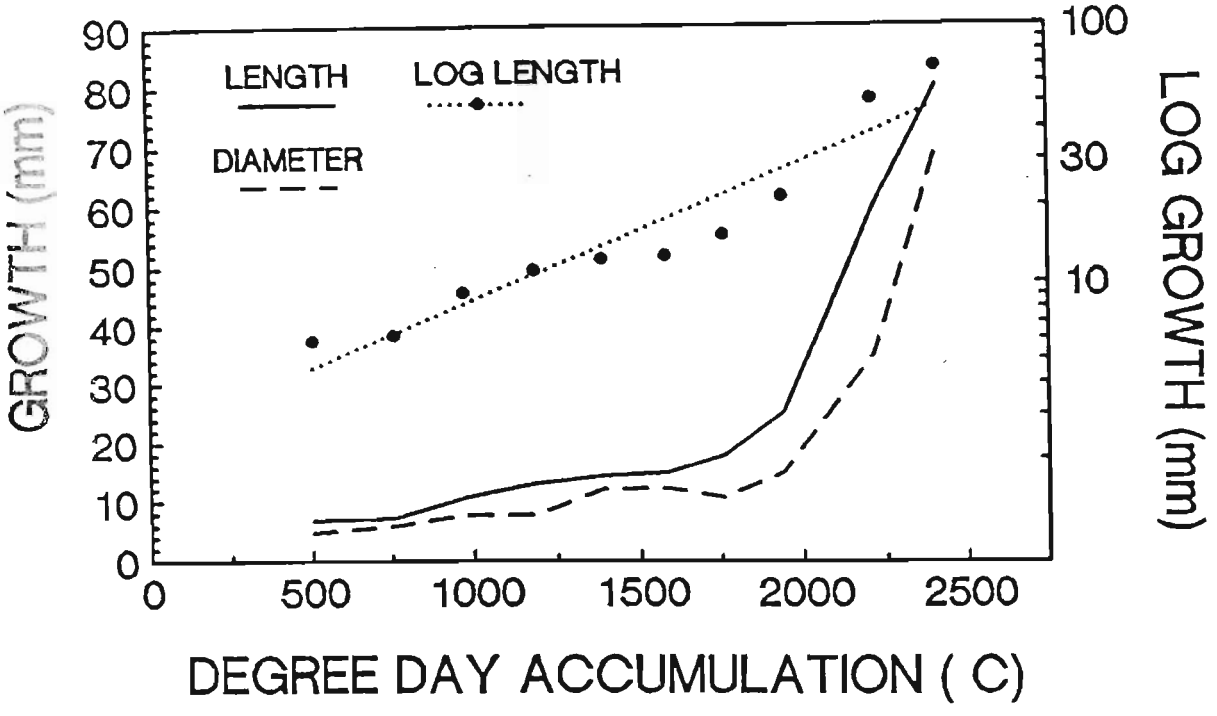


Figure 6. Dimensions of the flower bud plotted against degree day accumulation (^{o}C) over a base of 6^{o}C. September 1 represents the zero point at which accumulation began while 2400 units were accumulated by February 15. The regression plot of bud length on degree day accumulation is is nearly linear.

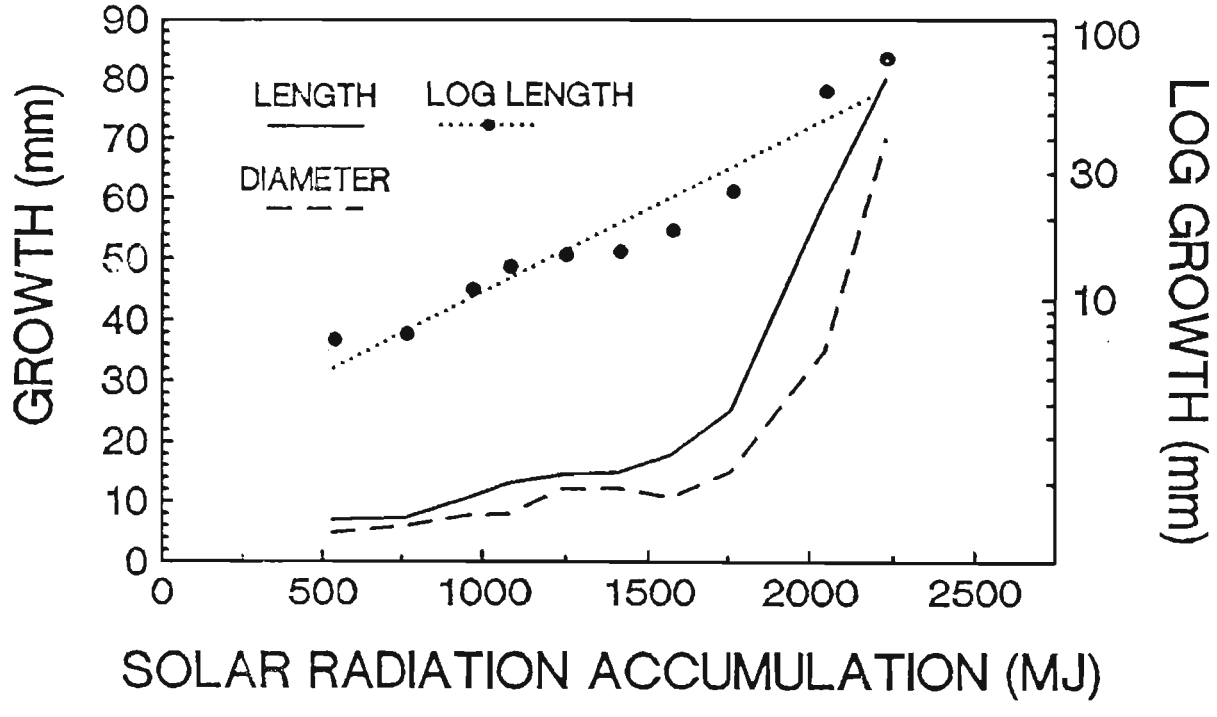


Figure 7. Dimensions of the flower bud plotted against daily solar radiation accumulation (MJ). September 1 represents the zero point at which accumulation began while 2225 MJ were accumulated by February 15. The regression plot of bud length on solar radiation accumulation is nearly linear.

FLOWER INITIATION IN PROTEA AND TELOPEA

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Abstract

Growth measurements of Protea neriifolia 'Salmon Pink' and Protea cynaroides 'Long Leaf' after flowering indicate multiple flushes of growth. Floral initiation occurs in P. neriifolia after the first spring flush. P. cynaroides may initiate flower heads only during two short periods after the two flushes each year. Floral primordia of Telopea speciosissima were examined using dissection and scanning electron microscope techniques.

1. Introduction

Protea neriifolia and Protea cynaroides seedlings flower only after passing through a juvenile phase. As seedlings grow through the juvenile phase, branching occurs and stems thichen, given good growing conditions. Root growth must occur rapidly to support both the shoot and future inflorescence development. A seedling of Protea neriifolia will produce its first flowers usually two or three years from planting. The Long Leaf variety of Protea cynaroides often produces one inflorescence three years from planting, although flowering occasionally occurs in two years. Plants of P. neriifolia grown from cuttings may flower as rooted cuttings if mature wood is present.

The Protea cut flower industry must depend on a sufficient supply of flowers with good quality blooms. The current market supply for many Proteaceous species varies considerably during the year with shortages of flowers at some periods and a glut at others. As part of understanding the control of flowering it is important to establish when flower initiation occurs in the annual cycle for each species.

The current work investigates the initiation of flowering in Protea neriifolia 'Salmon Pink', Protea cynaroides 'Long Leaf' and Telopea speciosissima. This has been done by dissection studies and electron microscopy.

2. Materials and Methods

Both vegetative and reproductive shoot tips of Protea neriifolia 'Salmon Pink' and the Long Leaf varieant of Protea cynaroides have been dissected. The 'Salmon Pink' selection has inflorescences which grow above the terminal leaves, and medium sized flowers. The color of the outer bracts is a soft pastel pink. The flowers show very little bract burning or curling. The clone originated in Victoria, where it flowers from April to November.

Shoot and flower tips were collected from 2 year old Protea neriifolia 'Salmon Pink' and Protea cynaroides and 4 year old Telopea speciosissima grown in the field at a plantation near Peats Ridge, New South Wales. After the autumn flowering season in 1988, five shoots on each of 20 Protea neriifolia 'Salmon Pink' and Protea cynaroides were labelled in May. During the middle of each month vegetative growth on each branch was recorded. Inflorescence bud development was noted on all plants. Floral buds collected from T. speciosissima shoots were dissected and all bracts removed. Resin moulds of the shoot tips were made by impressions using Spurr's resin. The floral apices were coated with platinum or gold and then examined under the scanning electron microscope.

3. Results

Both Protea showed a spring growth flush, with other flushes during the year (Figs. 1, 2). P. neriifolia 'Salmon Pink' grew with successive flushes during the summer, while P. cynaroides showed a significant reduction in growth during December (summer). Flower buds of P. neriifolia 'Salmon Pink' were found to be initiated from late October to early November at Peats Ridge. A receptacle begins to develop on the end of terminal shoots (Figs. 3, 4) which then develops into determinate flowers. Early during initiation a few flower buds abort with no flower. These shoots were observed not to develop further. By early December the flower buds had completed initiation and "bypass" or lateral shoots began to develop. In February some early flowers had black fur tips on the outer bracts.

Mature plants of P. cynaroides 'Long Leaf' developed flower buds during May with another initiation period in December. During both these times some bracts appeared to be remnants of floral buds which grew into vegetative shoots. A receptacle develops under the apical meristem as the stem widens. Bract development, flower head enlargement and elongation occurred over a 5-6 month period.

Shoot tips collected from Telopea speciosissima showed the first evidence of initiation in mid December (Figs. 5, 6). Floral buds developed more rapidly on older shoots as compared to the current season's growth. Floral primordia emerged from mid January to February. Pairs of florets differentiated as a new floral cone developed (Figs. 7, 8).

4. Discussion

Protea neriifolia 'Salmon Pink' has a definite cycle of growth and flowering. Flower initiation occurs just after the first main flush of growth in spring. Two further flushes of side shoots or small stems will occur given adequate moisture. The abortion of flower heads occurs only within a limited time, perhaps due to higher than normal temperatures or moisture relations at that time. During winter, shoots grow very little and flowering of the previous year's buds continues at a slow rate.

The initiation of flower heads in Protea cynaroides 'Long Leaf'

seems to occur for short periods twice a year. The apical meristem has the ability to set quickly or to revert to vegetative growth. Further research is warranted to determine how and why this occurs. It is not uncommon to find inflorescences with leaves which appear as bracts.

Telopea speciosissima has a primary flush of growth from November to January. Floral primordia initiate over a 6-8 week period after which there may be another vegetative flush of growth on the plants. A few plants flower in March and April which may mean that flowering may be changed given a certain stage of growth or conditions.

Acknowledgements

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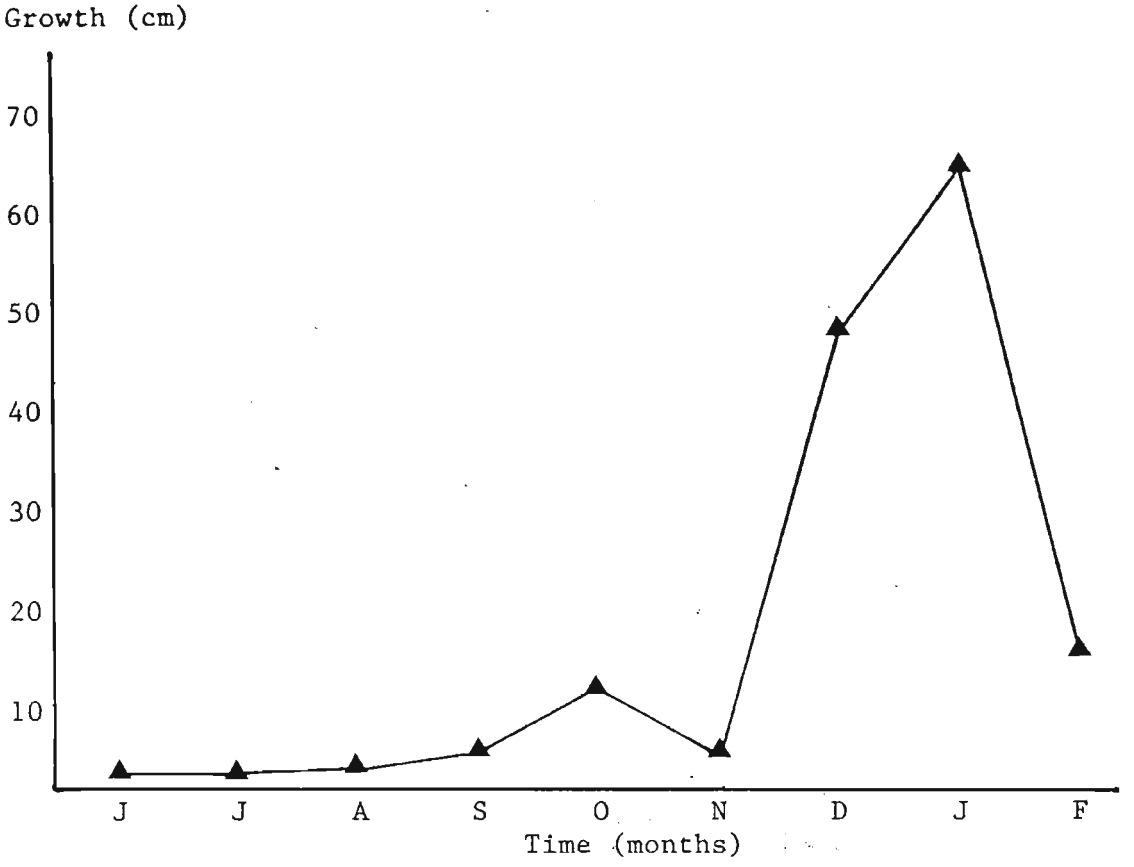

Figure 1 - Total amount of vegetative growth of Protea neriifolia 'Salmon Pink' on 20 two year old clonal plants.

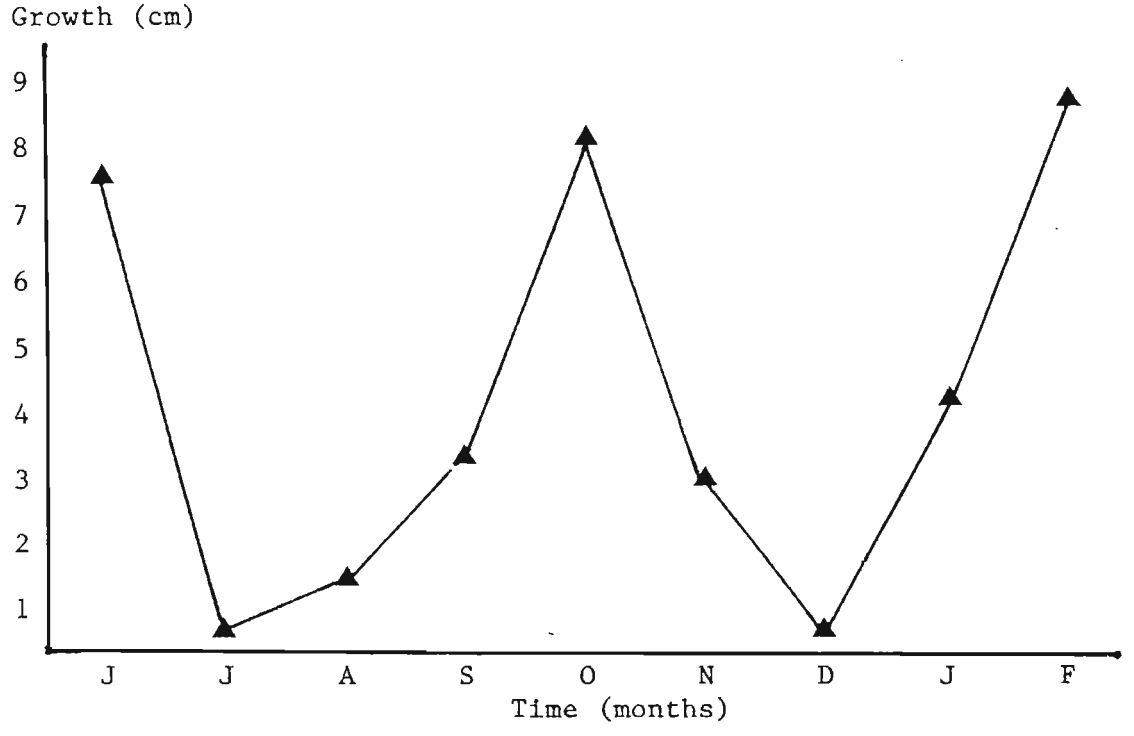

Figure 2 - Total amount of vegetative growth of Protea cynaroides 'Long Leaf' on 20 two year old seedlings at Peats Ridge.

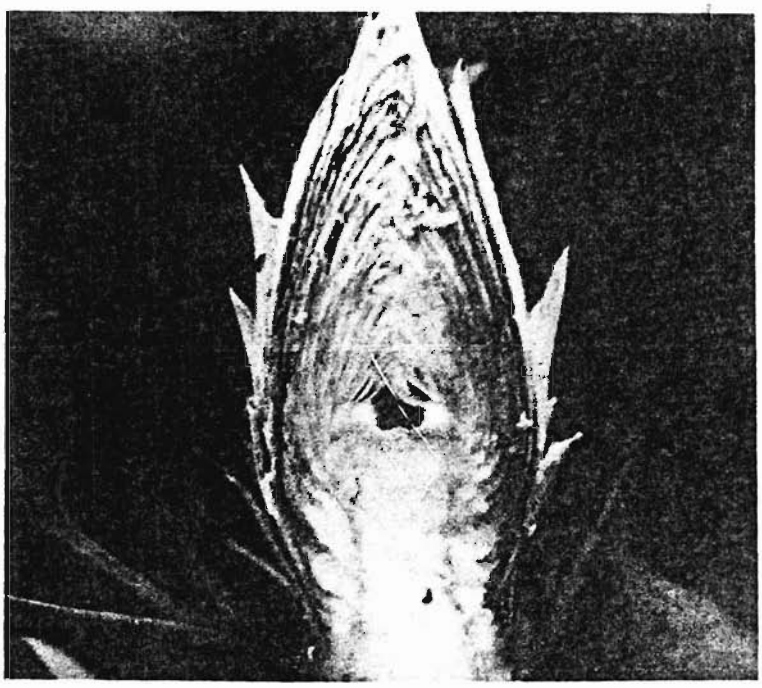

Figure 3 - Flower head of Protea cynaroides 'Long Leaf' variant with developing flowers on receptacle.

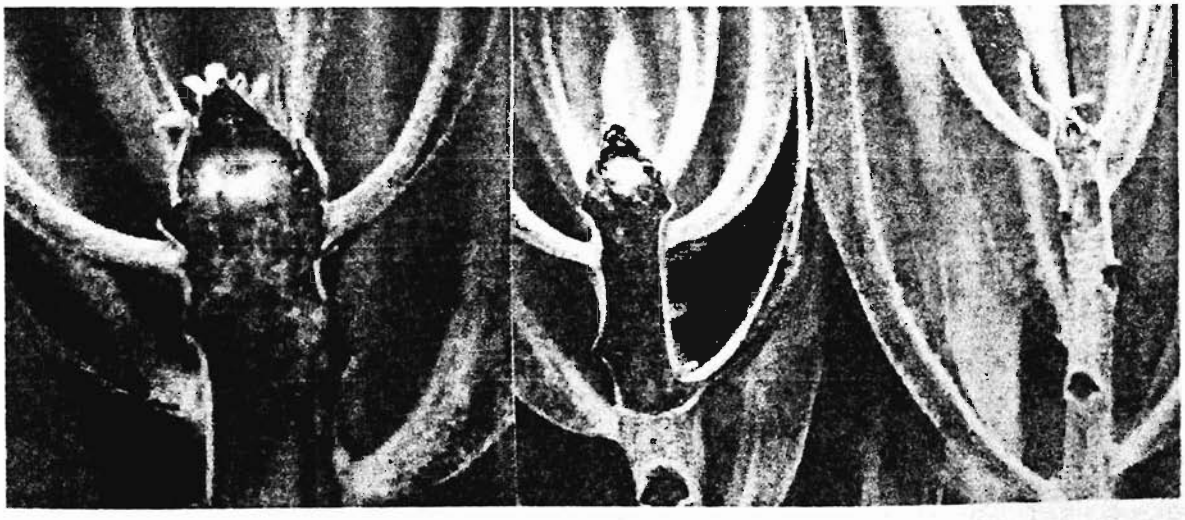

Figure 4 - Close up (left) and comparison of Protea neriifolia 'Salmon Pink' floral and vegetative shoots in early November at Peats Ridge, New South Wales.

Figure 5 - Scanning electron micrograph of the apical meristem of floral primordia of Telopea speciosissima.

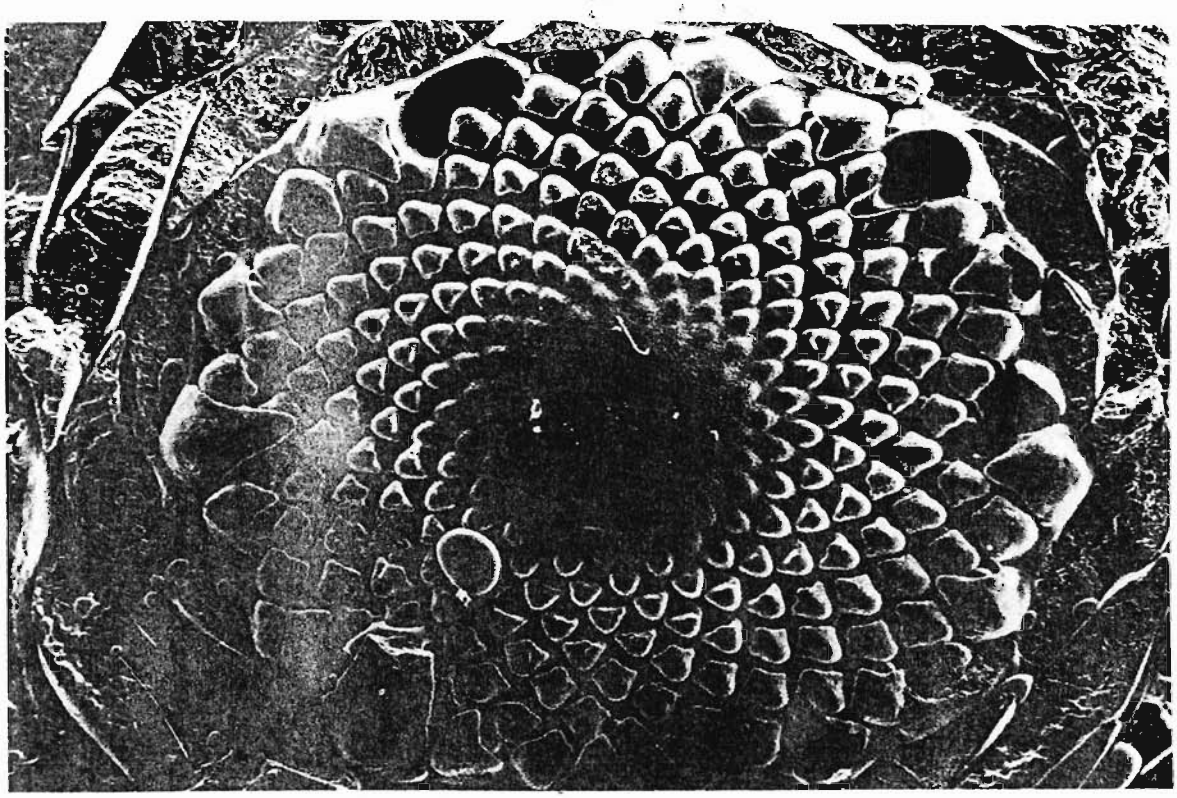

Figure 6 - Scanning electron micrograph of differentiating bracts and florets of T. speciosissima.

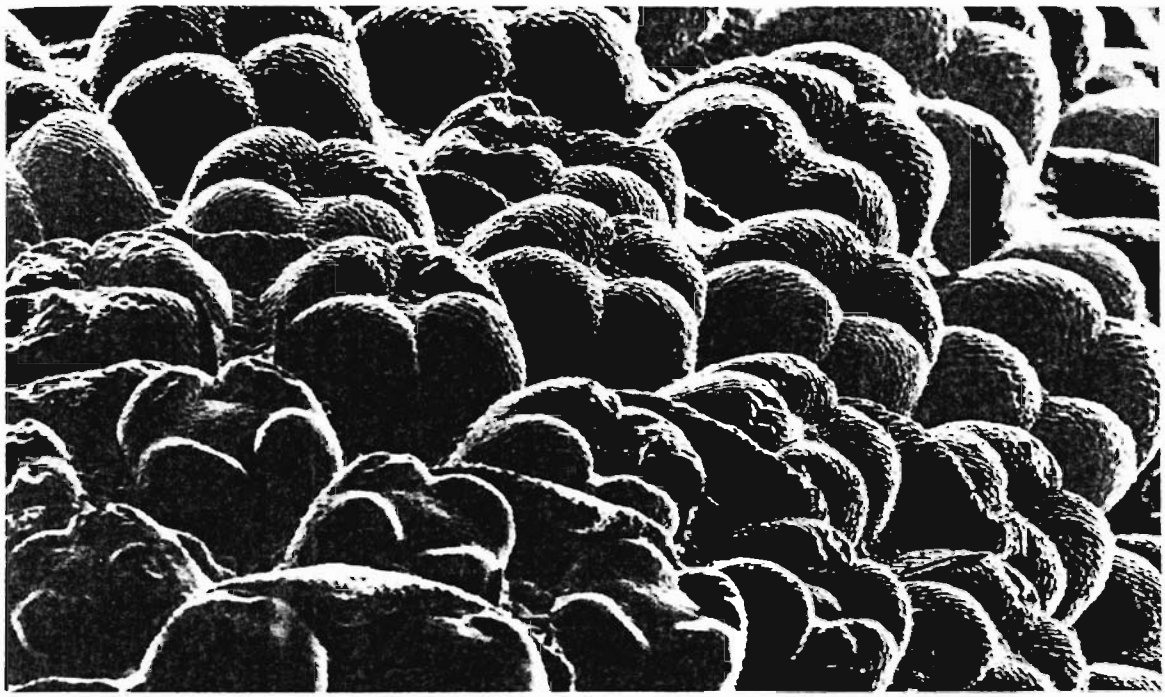

Figure 7 - Scanning electron micrograph of individual flowers developing in February on T. speciosissima apical meristem.

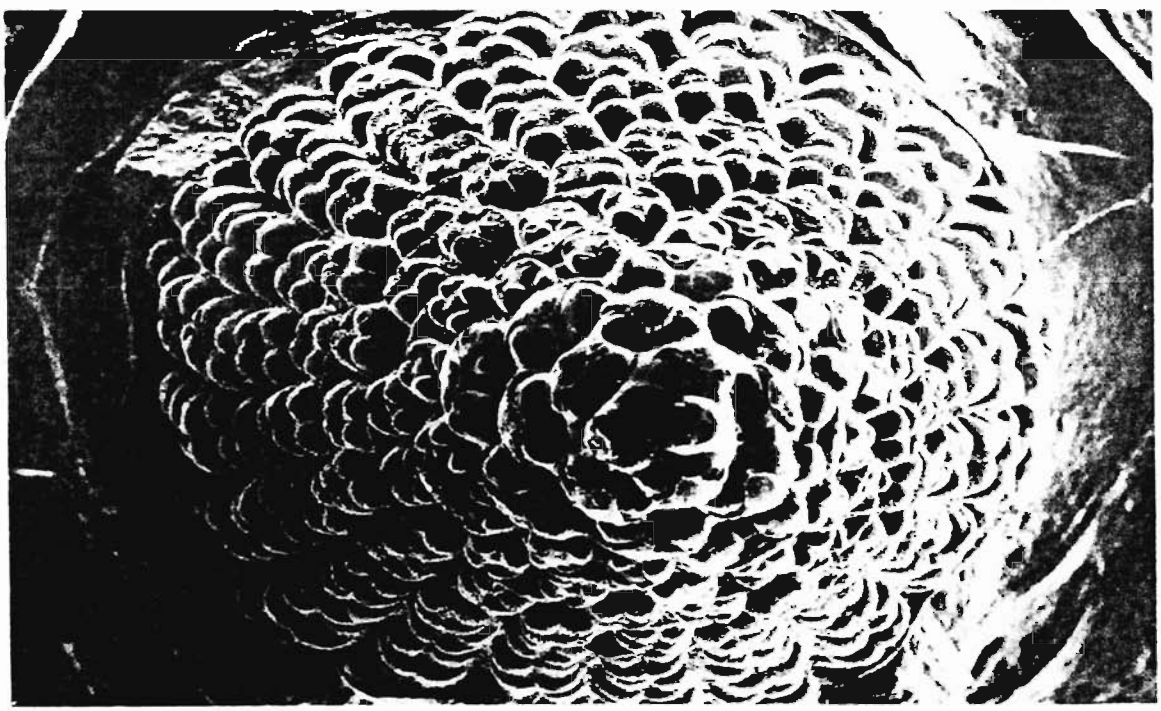

Figure 8 - Scanning electron micrograph of T. speciosissima floral head showing developing paired flowers.

EFFECT OF TEMPERATURE, DAYLENGTH AND GROWTH REGULATORS ON FLOWERING IN PROTEA, TELOPEA AND LEUCOSPERMUM

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Abstract

Flowering of 27 clonal plants of Protea neriifolia 'Salmon Pink' was recorded at each of four Protea plantations in southeastern Australia. Significant differences have been found in both period of flowering and numbers of blooms. Flowering of Telopea speciosissima was recorded on tagged plants at five field sites. A warm winter advanced and enhanced flowering more so in cooler areas. Treatments with gibberellic acid and cytokinins applied in July promoted early flowering in Leucospermum cordifolium. Disbudding delayed flowering by two to three weeks.

1. Introduction

Plants grow and flower at different periods of time in response to the environment in field produced Protea. The research described in this paper aims to establish a flowering date base for four locations and to determine what environmental factors determine the time of flowering in Protea neriifolia 'Salmon Pink', Telopea speciosissima and the Long Leaf variant of Protea cynaroides.

A separate experiment has been conducted to test the effects of disbudding and growth regulators on the flowering of Leucospermum cordifolium. Other researchers have found that flowering in other Leucospermum can be delayed by deheading (Jacobs and Honeyborne, 1978 and Brits, 1986) or by using ethephon and that growth regulators can alter flowering (Jacobs, Napier and Malan, 1986 and Halevy, 1983). The flowering of secondary inflorescences of Leucospermum was found to depend on the mean daily temperature (Jacobs and Honeyborne, 1979).

2. Materials and Methods

The experiments were conducted at five separate commercial Protea plantations in coastal New South Wales and southeastern Queensland. The geographical and soil data are given in Table 1. All the areas receive summer rainfall with spring being the driest period of the year. The maximum and minimum daily temperatures and precipitation recorded at each site.

2.1 Protea Phenology

During April, 1987, 27 one year old clonal plants of *Protea neriifolia* 'Salmon Pink' were planted at each plantation except site No. 2 in a complete random design. All plants were watered after planting and thereafter received only natural precipitation. Weedmat or mulch is used to control weeds. One year after planting, selective pruning of weak or low branches was carried out so that each plant had 12 to 22 branches at least 20 cm long. The flowering date of each inflorescence was recorded as the time when the inner bracts of each flower head began to separate at the top of the inflorescence. After flowering each inflorescence was pruned, removing as little of the stem as possible.

Table 1 - Location and Soil Type of Five Protea Plantations in Southeastern Australia

Characteristic	Plantation No. 1 Springbrook Queensland	No. 2 Taree, N.S.W.	No. 3 Peats Ridge N.S.W.	No. 4 Kurrajong N.S.W.	No. 5 Robertson N.S.W.
Latitude	28°14'S	31°52'S	33°19'S	33°30'S	34°35'S
Longitude	153°17'E	152°28'E	151°15'E	150°35'E	150°37'E
Altitude (m)	610	170	280	580	740
Aspect	southeast	north	northeast	east	east
Soil Type	reddish silty clay loam	brown sandy clay loam	yellow sandy loam	dark silty clay loam	reddish sandy clay loam
Soil pH	5.0	4.7	5.2	4.8	4.8

2.2 Telopea Phenology

Healthy 3-5 year old seedling *Telopea speciosissima* were chosen in April, 1987 to record flowring using metal labels. Plants were selected at random at all five plantations and observations of flowering were made during spring 1988. Each inflorescence was considered to be flowering when the first style was released on the flower head. Some dates were estimated due to time and distance factors. The inflorescences were picked by growers and stems cut back severely on each mother plant.

2.3 Growth regulator treatments

Ten flower buds on each of four plants of Leucospermum cordifolium were sprayed with a number of growth regulator treatments on 25 July 1988 between 10am and 1pm. All plants were at plantation No. 4 near Kurrajong Heights, New South Wales. Shoots with single dominant flower buds were treated at random on each plant.

The treatments consisted of: 1) a free flowering control 2) 250 mg/l of Grocel GA_3 (active ingredient: 100g/kg Gibberellic acid 3) 1.25 ml/l of Cytokin (GA_{4+7}) 4) 0.025 g/l of benzyl aminopurine (BA) 5) disbud the terminal bud of the shoot 6) 125ml/l of Bonzi (active ingredient: 4g/l Paclobutrazol) 7) 200 ml/1001 of Pro-Gibb (active ingredient: 100g/l Gibberellic acid and 8) disbud the terminal bud and spray with Cytokin (1.25 ml/l). Dates of flowering were recorded as the first style was released on each inflorescence.

3. Results

3.1 Protea Phenology

The flowering of Protea neriifolia 'Salmon Pink' is summarized in Figure 1. The first flower opened at Peats Ridge in March, 1988. The last flower to bloom was at Robertson during mid October.

Plants at Springbrook produced the most flowers as shown in Table 2. The average flowers per plant at Springbrook was 4.3 compared to 1.0 flower per plant at Robertson.

3.2 Telopea Phenology

Bloom periods for the Telopea speciosissima plants are given in Figure 2. First flowers occurred in Springbrook where plants had the greatest number of blooms per plant. Table 3 indicates that an uneven number of plants were observed at each site.

3.3 Growth Regulator Treatments

The flowering of each plant of Leucospermum cordifolium is shown in Table 4. The control flowered under natural conditions. In contrast, shoots with Grocel, Pro-Gibb and Bonzi partly became vegetative during November or ceased development. Disbudding the primary bud resulted in the most consistent delay in flowering.

4. Discussion

The flowering of Protea neriifolia 'Salmon Pink' shows highly significant differences in flowering between four commercial plantations in terms of number of blooms and the period of flowering. This clone generally flowers in autumn but due to cool winter temperatures flower rate of development slows considerably until September. It may be possible to alter flowering by either pruning or chemical means.

Further flowering data is required for Telopea speciosissima to determine the effects of climate from one year to another. The species produces many flowers within a very short period. This will cause more difficulty in attempts to extend the flowering period. However, some plants flower in autumn. The 1988 season was wet which increased bud rot in both plantations 1 and 2. The warm winter advanced flowering more in the southern districts. Inflorescence development should not be related to daylength whereas flower initiation may be.

Leucospermum cordifolium flower buds do respond to some growth regulators by promoting or delaying flowering. Both cytokinins and ProGibb applied to flower buds advanced flowering by one to three weeks. Disbudding may be used to delay flowering by two to three weeks but is less effective as the spring temperatures increase.

Acknowledgements

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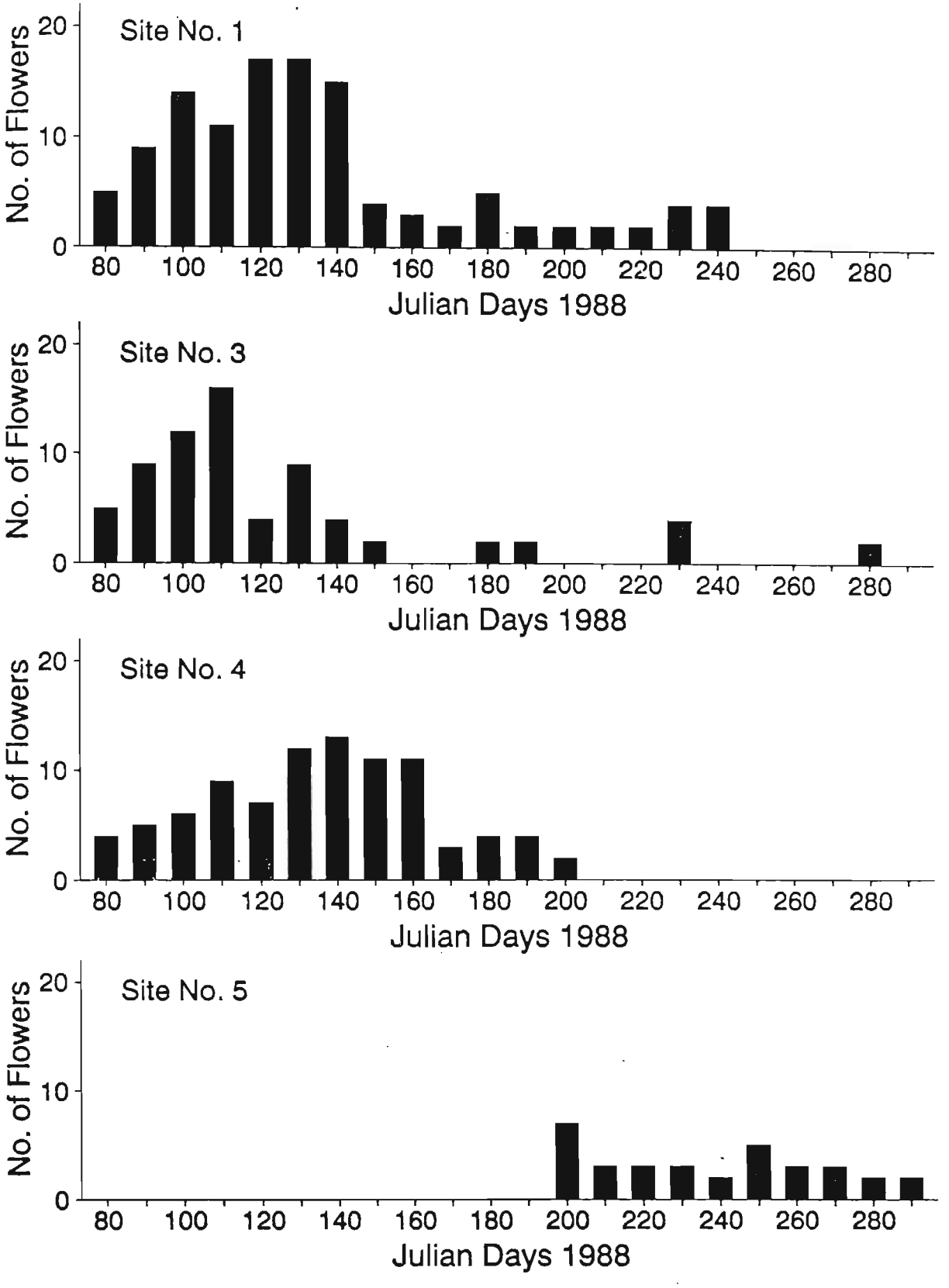

Figure 1. The flowering of Protea neriifolia 'Salmon Pink' at four protea plantations during 1988.

Table 2 - Data Analysis of *Protea neriifolia* 'Salmon Pink' flowering for 1988.

Site	Mean in Julian D.	S.E. mean	Number of Flowers	Flower size(mm)	Abortions
1	124	3.21	112	124	2
3	113	4.30	68	116	3
4	132	2.78	90	121	2
5	232	5.81	26	108	5
Total			296		12

Table 3 - Comparison of Flowering of *Telopea speciosissima* at five sites in southeastern Australia

Site	Mean in Julian D.	S.E mean	Number of Plants	Number of Flowers	F Value
1	250	6.77	25	249	15.45
2	268	9.30	24	133	16.84
3	257	5.25	57	249	5.12
4	266	4.89	48	249	14.69
5	269	4.88	57	268	6.97

Grand Flowering Mean: 263

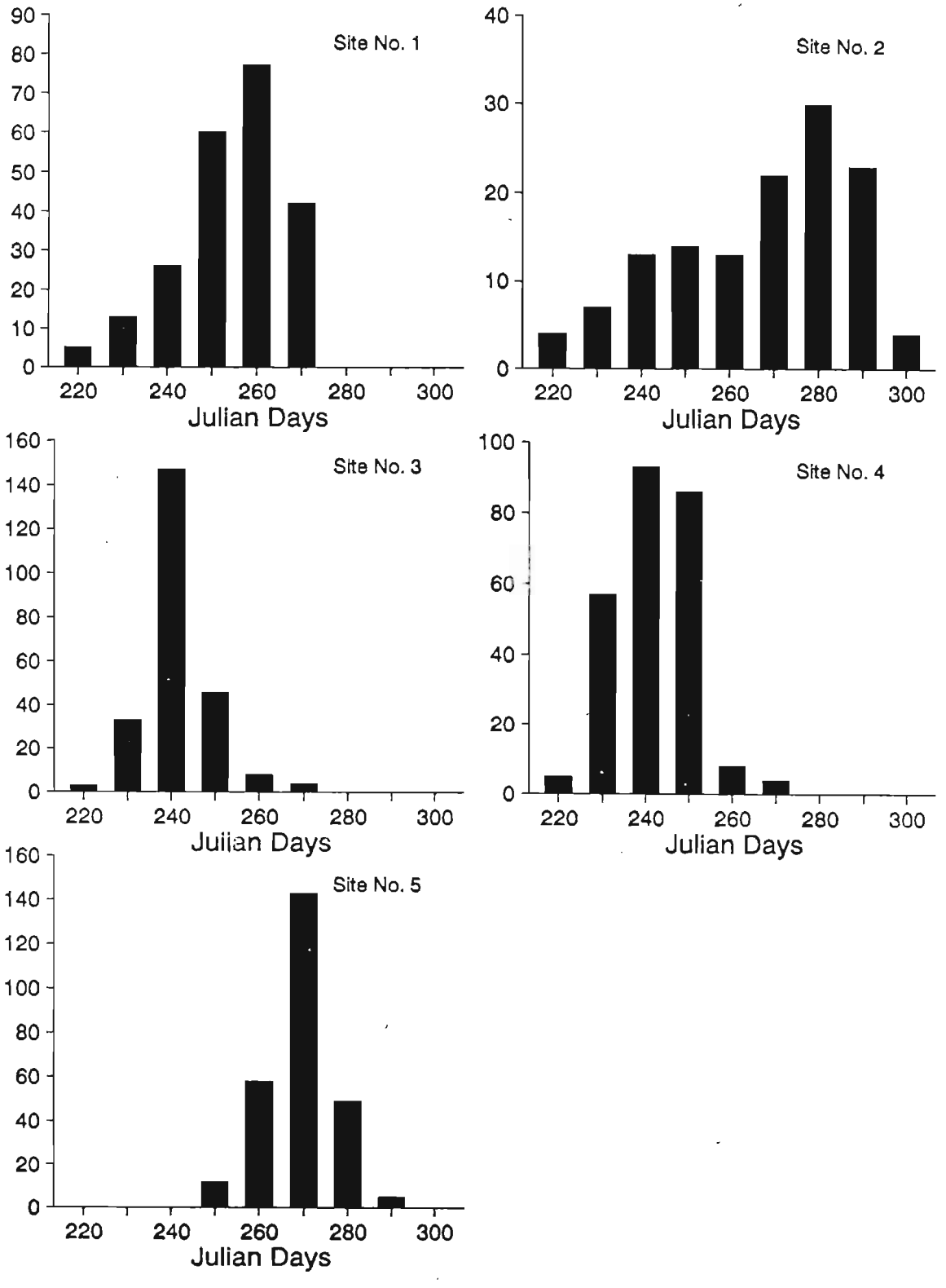

Figure 2. The rate of flowering of Telopea speciosissima at five protea plantations during 1988.

Table 4 - The effect of growth regulators on flowering of *Leucospermum cordifolium*.

	Days of Flower of all Plants				S.E.
	Plant Number				
Treatment	1	2	3	4	
1	292	304	298	291	2.95
2	280	298	300	293	3.77
3	285	290	285	288	3.70
4	287	298	287	284	3.45
5	303	301	308	305	3.42
6	296	301	298	266	4.02
7	292	289	281	285	3.73
8	290	293	287	282	3.66

FLOWER STRUCTURE AND THE INFLUENCE OF DAYLENGTH ON FLOWER INITITATION OF SERRURIA FLORIDA KNIGHT (PROTEACEAE)

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Abstract

Reproductive development of Serruria florida commences during March with the terminal inflorescence slowly increasing in weight while the apical meristem is producing peduncular bracts (pb). Following pb initiation the growth rate increases, the apical meristem enlarges and produces 30-40 bracts of which 10-15 abort shortly afterward. Incandescent light applied from 5 March 1988 from 6 pm to 8 am prevented flower formation indicating that the natural short days of winter are required for flowering.

Introduction

Serruria florida is commercially grown as cut flower under winter rainfall conditions in the Republic of South Africa. The major problem in marketing the crop is a very short flowering period. The development of the genus Serruria as flowering pot plants is also hindered by an inability to control its flowering time. The plants grow vegetatively during spring and summer. Reproductive development commences during autumn after extention growth has terminated, and flowers open during July and August.
The purpose of this study was to investigate the morphological development of the conflorescence and the role of photoperiod in induction of flowering.

Materials and methods

Plant material. Serruria florida plants grown in a commercial plantation were used. The plants were 4 years old and were propagated from seed. The plants were clean cultivated and not irrigated or fertilized. The mean annual rainfall for the area is 600-700 mm. The plants were interplanted between Protea cv. Carnival and spaced 1,5 m in the row and 3,5 meters between rows.

Conflorescence structure. Twenty shoots were randomly selected on 13 July and the following determined: the

number of inflorescences/shoot, the position of the inflorescences on the shoot, shoot diameter, the length of the peduncle, the number of peduncular bracts and the number of florets of the terminal inflorescence from each shoot.

Inflorescence development. The developing terminal bud of 60 randomly selected shoots (30-50 cm long) was collected every fortnight from 21 March to 5 July. The fresh weight of each bud was determined and the 10 buds best representing the mean fresh weight were used for a SEM investigation. The remaining 50 buds were randomly devided into 5 groups with 10 buds/group, dried for 7 days at 80°C and their dry weight determined. The buds used for the SEM investigation were prepared and photographed as described by Malan[1].

Effect of photoperiod on floral initiation. Two incandescent light bulbs (100 Watt) with reflectors were suspended 80 cm apart 30 cm above each of 6 plants. The light intensity measured with a light meter (Lambda Instruments Corp Photometer with a PAR sensor) was 15 μ mol $m^{-2}s^{-1}$ 60 cm from the light source. The lights were on from 6 pm to 8 am from 5 March. On 5 March, 22 March and 19 April 12 shoots were decapitated by 5 cm. Shoots decapitated on the same dates on plants grown under natural conditions served as controls. The number of shoots forming an inflorescence was counted on 13 July. Lights were switched off above 2 individual plants on 3 May and 30 May.

Results and discussions

The conflorescences of *S. florida* (Fig. 1) consisted of 1-11 capitula (Fig. 2) borne distally on the shoot on 3-4 cm peduncles. The number of capitula on a shoot is dependent on shoot diameter with thicker shoots bearing more inflorescences. The lower probability of inflorescences occurring at node no. 2 and 3 is possibly due to correlative inhibition by the terminal inflorescence.

The development of the inflorescences were initiated during early March. The dry weight of the terminal inflorescence initially increased slowly but after 19 April the dry weight increased rapidly (Fig. 3). The terminal inflorescences reached anthesis on approximately 5 July followed by the axillary inflorescences which reached anthesis approximately simultaneously.

During the slow early development of the bud bract primordia were initiated (Fig. 4A). Approximately the first 10 primordia developed into peduncular bracts (Fig. 2). Peduncular bract initiation was followed by a flattening of the apical meristem and the rapid initiation of 30 to 40 bract and floret primordia (Fig. 4B and 4C). Approximately 15 of the distal florets aborted at a very early stage with only approximately 25 florets developing to anthesis.

Floret initiation was completed and perianth initials could be distinguished on the florets by 4 May (Fig. 4D). Hairlike structures which developed on the perianth and bracts obscured the florets (Fig. 4D) preventing a continuation of the SEM investigation.

Vegetative development of completely inhibited buds after shoot decapitation on all shoots exposed to continuous light (LD) while those on control plants developed reproductively confirms the role of short days in induction of flowering of Serruria florida.

Flower development was normal on the plant above which artificial LD were discontinued on 3 May and terminal flowers reached anthesis by mid-September. Axillary flowers reached anthesis 2-3 weeks later. On the plant above which LD was discontinued on 30 May peduncular bracts were green and forked like foliage leaves, the involucral bracts were green and the flowers were not marketable.

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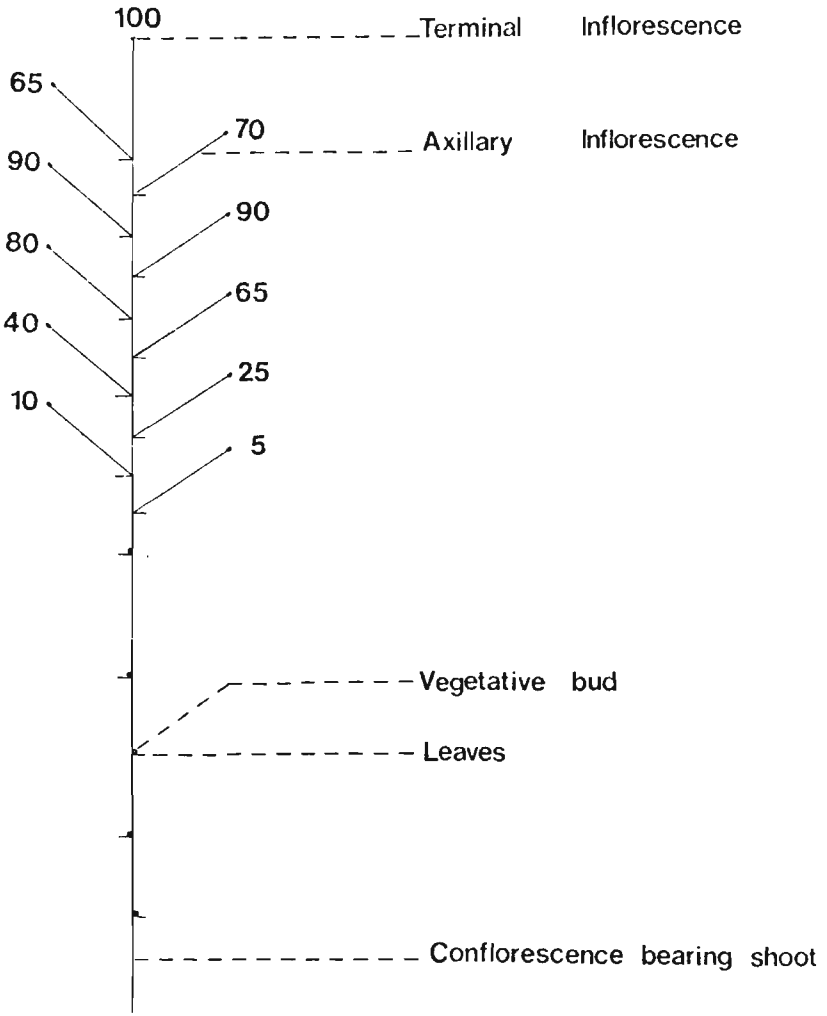

Fig. 1: Schematic representation of a flowering shoot of Serruria florida. The values next to each node position represent the % probability of an inflorescence occurring at such a node based on the average of 20 randomly selected shoots.

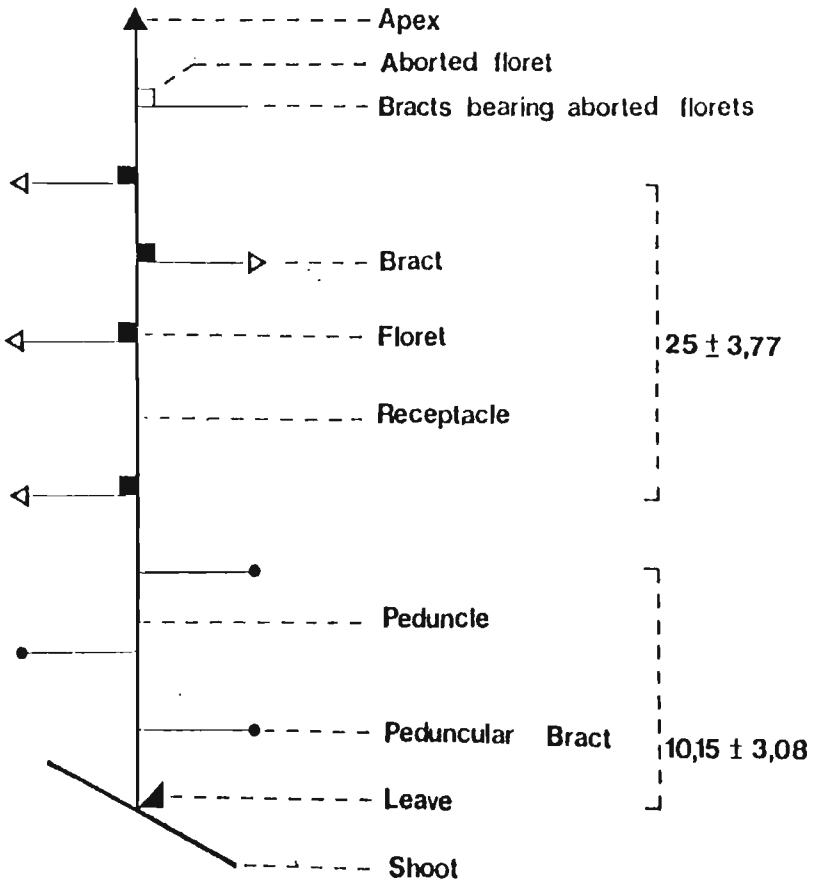

Fig. 2: Schematic representation of the apical inflorescence of Serruria florida. The numbers on the right represent the number of bracts/area based on the average of 20 terminal inflorescences.

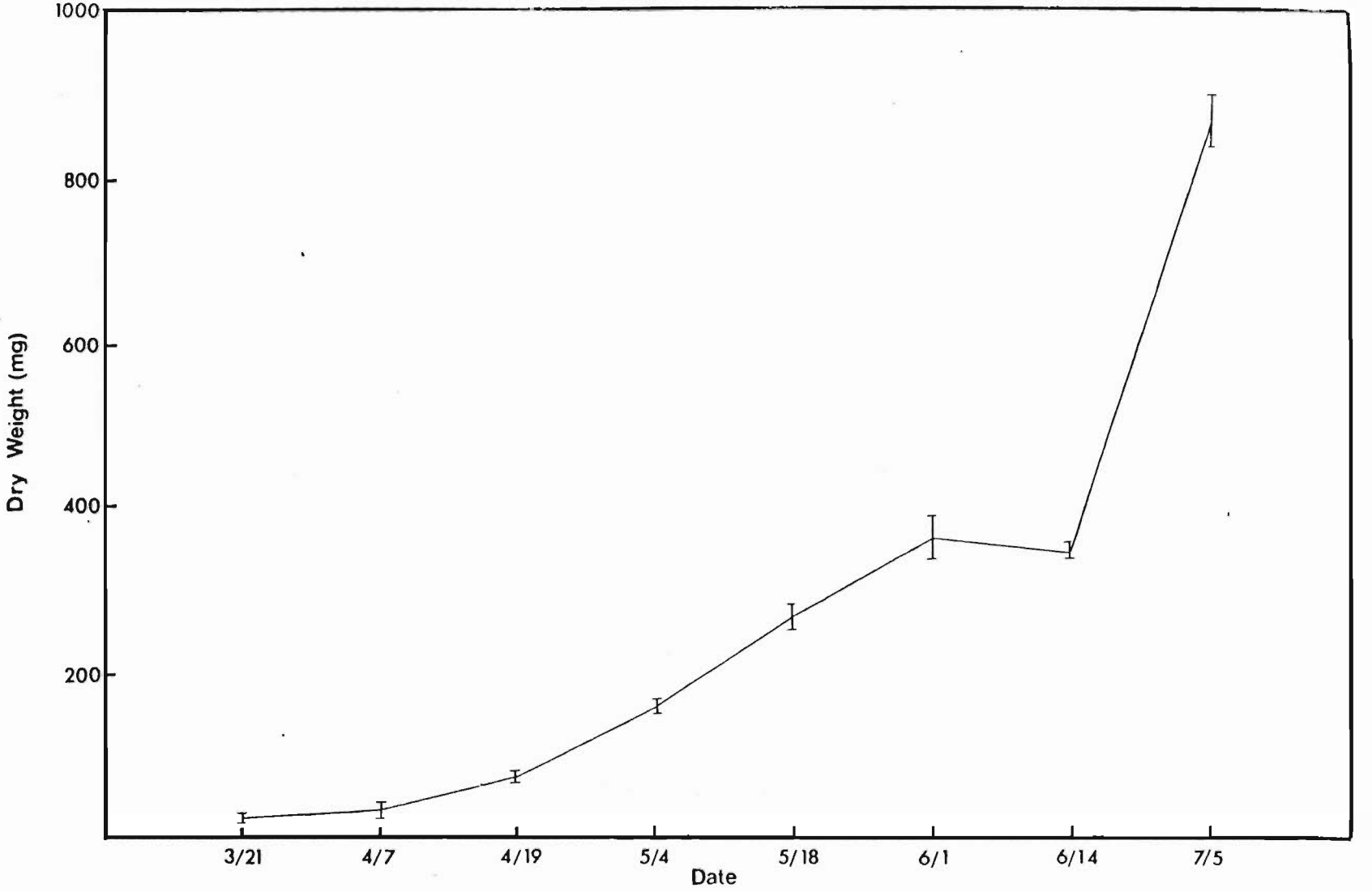

Fig. 3: The increase in the dry weight (mg) of the terminal inflorescence of S. florida. Values based on the average of 5 treatments with 10 inflorescences per treatment.

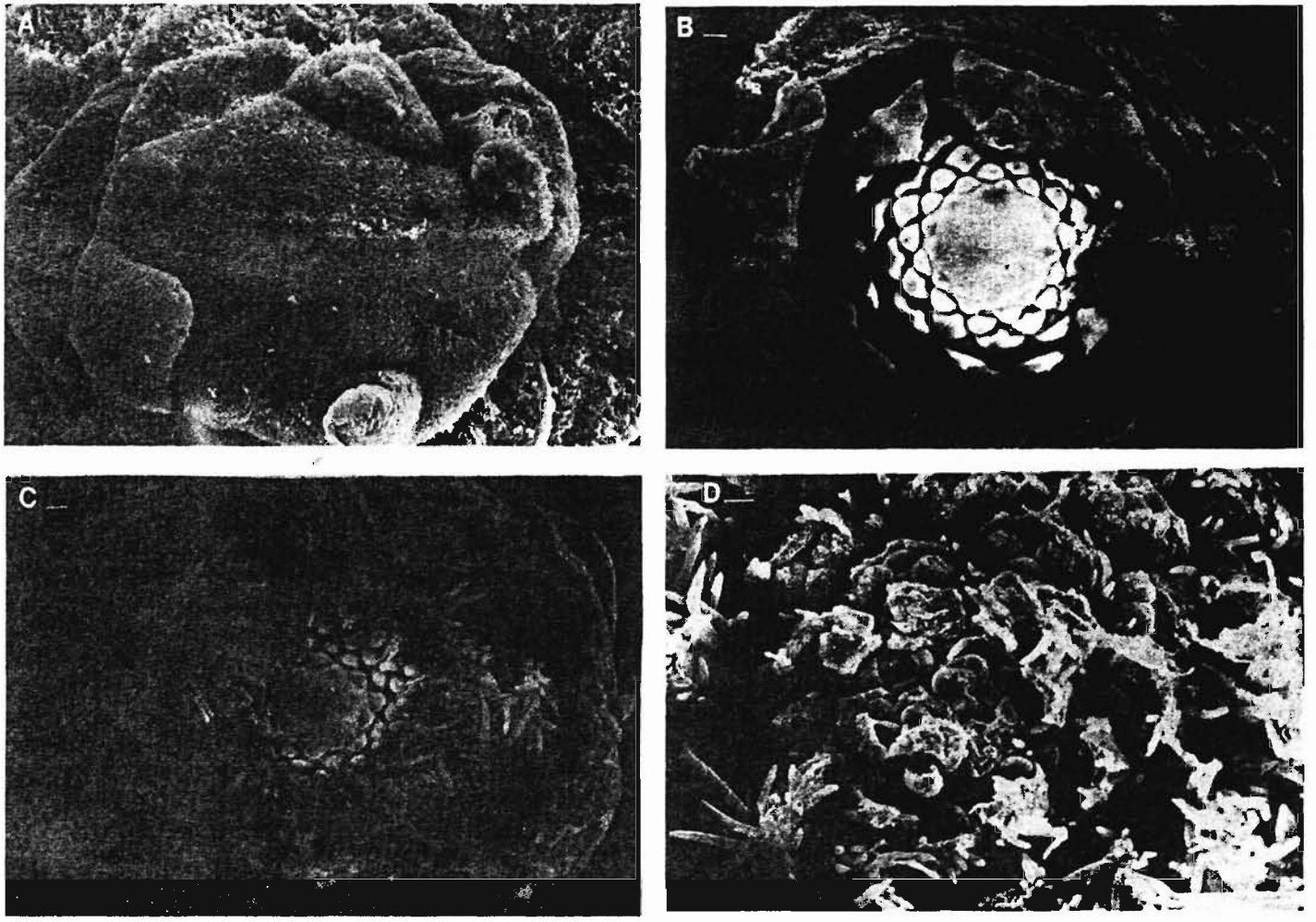

Fig. 4: Development of the terminal inflorescence of Serruria florida (SEM, bar = 100 µm). **A.** Apical meristem (on 3/2) initiating involucral bracts. **B** and **C.** Apical meristem (on 4/7 and 4/19 respectively) initiating bract and floret primordia. **D.** Inflorescence (on 5/4). Floret initiation completed. Perianth initials visible on florets. Hair-like structures on bracts and perianth obscure basal florets.

PROTEA POSTHARVEST BLACK LEAF A PROBLEM IN SEARCH OF A SOLUTION

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Abstract

It has been proposed that postharvest blackening of protea (*Protea neriifolia*) leaves is caused by water stress and/or insufficient carbohydrate supply. The following research was undertaken to determine the relative contribution of each to leaf blackening. The rate of development of leaf blackening varied with clonal source, season, and developed sooner in flowers harvested at the closed bud stage, with the slowest rate occurring in flowers harvested when the flower bracts had just started to unfold. Leaves were slower to blacken if harvested in the afternoon than in early morning. The immediate removal of field heat from flowers was only worthwhile if low temperature was maintained to the wholesale or retail level. High light intensities postharvest, solutions containing sugar, flower girdling and removal, significantly delayed the onset of leaf blackening. Water uptake by flowers rapidly declined after harvest with the commercial preservative solutions delaying the rate of decline in uptake. The decline in water uptake paralleled the loss in flower fresh weight. Preharvest irrigation regime did not significantly influence postharvest leaf blackening rate. The effect of flower carbohydrate supply on leaf blackening was felt to be modified by flower stem water uptake ability.

1. Introduction

Leaf blackening or browning of Protea flower, especially *Protea eximia* and *P. neriifolia*, is a serious problem postharvest (Ireland *et al.*, 1967). The leaf blackening leads to loss of decorative value, loss of market value and possible rejection of the consignment. A solution to this problem has not yet been achieved. Leaf removal is difficult without inflicting considerable damage to the stem because of the sessile nature of the leaves (Paull *et al.*, 1980).

The symptoms of this leaf disorder have been described many times (De Swardt *et al.*, 1987; Paull *et al.*, 1980; Brink and De Swardt, 1986; Ferreira, 1983; Haasbrock *et al.*, 1973). Leaf blackening have been divided into four types (De Swardt *et al.*, 1987) based upon the first appearance of the discoloration. The blackening probably occurs because of oxidation of phenols (Van Rheede van Oudtshoorn, 1963) and leuco-anthocyanins (Elsworth and Martin, 1971), that are found in protea leaves. The lack of relationship between these substrates, polyphenoloxidase and rate of leaf blackening (Ferreira, 1983) is not surprising

since the concentrations and activities are very high. The intensity and rate of symptoms development varies from year to year (De Swardt *et al.*, 1987), within a season and between different clones of one species (Akamine *et al.*, 1979; Paull *et al.* 1980).

Knowing what causes the blackening and regarding it as an abnormal senescence phenomenon have not enabled researchers to determine the trigger. Two major hypotheses are in vogue. One regards the leaf's water status as the trigger (Paull *et at.*, 1980; Ferreira, 1983; Mulder, 1977), with the other possible trigger being depletion of intracellular reserves possibly sugars (Brink and De Swardt, 1986). The relative importance of each may depend upon the actual circumstances.

Protea have been grown in Hawaii for some years (Parvin *et al.*, 1973). The postharvest handling system in Hawaii of protea places additional constraints on alternatives. Flowers are normally picked in the morning and packed either that day or within 24 hours in cardboard boxes. The boxes are all shipped by air without refrigeration. Hence, a solution in which refrigeration is not part of the handling system is desirable. The following work was undertaken to define the preharvest factors that lead to more rapid leaf blackening, then to attempt to reduce the rate of blackening by altering the rate of water loss and by providing sugars.

2. Material and Methods

Terminal shoots with flower buds of *Protea neriifolia* were either cut from several clones at the Maui Agricultural Research Center at Kula, Maui or obtained from a single commercial grower in the Kula area of Maui. The flowers were immediately boxed and air shipped to Honolulu where the investigations were carries out. The elapsed time from picking to initiation of an experiment was about 8 hours.

At least five stems were used for each treatment and all tests were repeated at least twice. Evaluation of the rate of leaf blackening was based upon the relative area of leaves with darkened surface as a percentage of total leaf surface area. Observation were recorded every two days until all the leaves were black. The time from harvest to 50% leaf blackening was used to calculate vase life. Observation on the center bracts of the flower were made for wilting and blackening. Vase life was evaluated under the following conditions: 20° to 25°C, 60 to 80% relative humidity, 10 hours fluorescent light (1 watt m^{-2} day^{-1}). All solutions were prepared in deionized water. Stems were recut following simulated shipping and prior to placement in solution.

3. Results and Discussion

3.1 Preharvest

Cultivar differences in rate of leaf blackening were reported for *Protea eximia* (Paull *et al.*, 1980), the differences being more pronounced when the flowers were held in commercial preservative solution. There was a three-fold

difference in the rate of leaf blackening between different clonal selections of *P. neriifolia* (Table 1). This clonal difference was not apparently associated with the different seasonal pattern of bush flowering. The Pink Splash clone peak flowering occurred between October through December, with Rose Mink in the November to January period and Pink Mink between July to November. Early season harvested flowers tended to be more prone to leaf blackening (Table 2). At the September-October period of the year the weather tended to be hotter and drier than later in the season.

Stage of flower opening at harvest significantly influenced days to 50% leaf blackening and days to moderate bract curling (Table 3). Leaves of flowers harvested in the closed bud stage blackened more rapidly than those harvested when the bracts were just unfolding or at a later stage. The optimum stage of harvest was when the bracts had just started to unfold as these flowers had longer total life than flowers picked at other stages.

Flowers harvested from part of a field of Pink Minks that had been irrigated at three week intervals did not show any significance in days to 50% leaf blackening and moderate bract curling from another part of the field receiving regular weekly irrigation. Flowers harvested in the early morning (8 am) had a shorter period to 50% leaf blackening than those harvested at 2 pm. Ferreira (1986) reporting the works of Muller (1977) indicates that an excessive loss of water was needed to initiate leaf browning. The above results do not fully support this conclusion.

3.2 Water Relations

The loss of water occurs both from the flower head and the leaves (Paull *et al.*, 1980). Water loss from the flower varied from 25 to 50% of the total. Removal of the flower head delayed leaf blackening (Table 4) as had been previously reported (Paull *et al.*, 1980). Girdling of stem just below the flower head also delayed leaf blackening to the same extent. This girdling result suggested that the effect of water loss from the head was not always the major factor initiating leaf blackening. The possibility of something being transferred from the flower head to leaves cannot be discounted.

Coating the leaves with various antitranspirants (Table 5) delayed leaf blackening though, the effect was not as marked, as the effect of flower head removal. The delay caused by antitranspirants was significant and repeatable.

Water uptake rate declined from ca. 150 ml day^{-1}, after recutting following 2 days packing, to 12 ml day^{-1} 6 days later. The rate of decline was less in flowers held in Florever (20 g l^{-1}), the uptake rate being 36 ml day^{-1} 6 days after recutting following 2 day pack. This agrees with our previously reported results (Paull *et al.*, 1980). De Swardt *et al.*, (1987) have suggested that a moisture loss of only 1% may be sufficient to initiate browning. It is unclear how this 1% loss relates to leaf water status. Akamine *et al.* (1979) only found a limited correlation between leaf dry weight and rate of leaf darkening, while Ferreira (1983) did find a significant relationship. Relative water content declines as leaf blackening increases (Figure 1). The relationship between

relative water content and leaf blackening did not answer the question as to whether water status was the trigger. Florever (20 g l^{-1}) delayed the decline in relative water content and rate of leaf blackening. These preliminary results have to be repeated.

3.3 Carbohydrate Relations

Use of preservatives in the vase solution reduced the rate of leaf browning, with sucrose being the most effective component (Akamine *et al.*, 1979; Brink and De Swardt, 1986). Exposure of flowers to high temperatures would stimulate respiration (Ferreira, 1986) and therefore deplete carbohydrate reserves, though water loss from these flower would be difficult to control. Pulsing of *P. neriifolia* flowers with greater than 7.5% sucrose was harmful and accelerated leaf browning. Brink and De Swardt (1986) indicated that a concentration higher than 1% was harmful yet a summary of Brink's PhD thesis (Brinks, 1987) stated 10%. The reason for the discrepancy is unclear. Our results support the 10% figure, however we were not able to greatly delay leaf blackening by pulsing with 10% w/v sucrose for 24 hours followed by 2 days pack.

Exposure of flower to high light had a significant effect in delaying leaf blackening (Table 6). Fluorescent light exposure (9 mE m^{-2} sec) was found to be inadequate in delaying leaf blackening. Increasing the period in simulated packing to 3 or more days significantly reduced the time to 50% leaf blackening (Table 7). These results suggest a significant role for light.

Brink's (1987) data is very useful in ascertaining where vase supplied carbohydrate end up in the cut flower. The results indicate that with longer periods (18 hours) of sucrose exposure, the flower head is the preferred sink (Brink and De Swardt, 1986). Leaves do receive significant amount of sucrose if the pulses are less than 12 hours (Brinks, 1986). He recommended a 12 hour pulse. Attempts to pulse flowers with sucrose concentration from 2.5% to 20% have not be[illegible] greatly encouraging. Concentration of 10% and greater have lead to leaf injury within 24 hours. The injury has taken the form of a marginal leaf water soak tissue appearance and premature leaf blackening. Lower concentration did marginally reduced the rate of browning. The significant difference from Brink's (1987) research method was that we subjected the flowers to a 2 days simulated packing.

4. Conclusion

The darkening of *Protea neriifolia* can be apparently triggered both by water stress and possibly a carbohydrate or other reserve shortage in the leaves. However, the role of the flower head in leaf blackening did not appear to be solely because of its role as a sink. Head removal and flower head girdling gave a similar delay in leaf blackening. It is possible that the head may contribute directly to initiation of leaf blackening.

These two triggers of leaf darkening might operate by their effect on

cellular membranes as suggested by Ferreira (1983). Attempts to measure this change would be difficult because of the high concentration of phenols present in the leaves that would be released during analysis.

The difference in rate of leaf blackening of flowers subjected and not subjected to a simulated packing period makes interpretation of some published data difficult. It would be worthwhile to know the underlying initiation factor in leaf blackening, but this should take into account commercial handling conditions.

The long term solution to this problem is to select clones that show less leaf blackening. Short term solutions are needed and these require an understanding of the preharvest factors that predispose some harvests to more rapid leaf blackening. The types of browning may be different with the tip, marginal and spot browning being one type and the wet steamy black leaf conditions found for some flowers on unpacking another.

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Table 1 Cultivar difference in number of days to 50% leaf blackening and to moderate flower bract curling for *P. neriifolia* flowers preconditioned overnight in Florever (20 g l^{-1}), packed dry for 3 days at 22°C and evaluated in Florever.

Clone	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Pink Splash	10 c	8 c
Rose Mink	24 b	35 a
Satin Mink	26 ab	16 b
Pink Mink	30 a	32 a

* Means in each column followed by the same letter do not differ statistically (P=0.05 Duncan/Waller Multiple range test).

Table 2 Seasonal changes in number of days to 50% leaf blackening and to moderate bract curling of *P. neriifolia* clone Pink Mink flowers preconditioned overnight in Florever (20 g l^{-1}), packed for 2 days at 22°C and evaluated in Florever.

Date of Harvest	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Sept. 22nd	17 c	12 c
Oct. 12th	34 a	22 a
Nov. 1st	28 b	18 b

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 3 Effect of stage of Pink Mink flower opening on days to 50% leaf blackening and moderate bract curling. Flowers were preconditioned in water overnight, packed for 3 days at 22°C and evaluated in deionized water.

Stage of Flower Opening	Days to 50% Leaf Blackening	Days Bract Curling
Closed Bud Stage	6 c	14 c
Bracts Just Unfolding	8 a	18 a
Cylindrical Shape	9 a	15 b
Conical Shape	8 a	14 c

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 4 Effect of flower removal and girdling the stem just below the flower on days to 50% leaf blackening of flowers held continuously in Florever (20 g l^{-1}).

Treatment	Days to 50% Leaf Blackening
Control	18 b
Flower Removal	24 a
Flower Girdle	24 a

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 5 Effect of antitranspirants on days to 50% leaf blackening of Pink Mink flowers preconditioned overnight in water after antitranspirant application, packed for 3 days at 22°C, and evaluated in water.

Treatment	Days to 50% Leaf Blackening
No Treatment	10 cd
Spray ABA 500ppm	11 bc
FMC-819 2% w/v	8 e
Folicote 5% v/v	12 ab
Polytrap 1% v/v	11 bc
Exhalt 20% v/v	12 a
Wilt Pruf 20% v/v	11 bc
Semperfresh 2% v/v	9 de

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 6 Effect of holding Pink Mink flowers continuously in light or darkness on rate of leaf blackening and to moderate flower bract curling. Flowers were held in deionized water.

Treatment	Days to 50% Leaf Blackening	Days to Moderate Bract Curling
Dark	10 b	11
Light (26 mE M^{-2} Sec^{-1})	16 a	11

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

Table 7 Flowers were held for 24 hours in Floralife packed dry for various length of time then evaluated in water for days to 50% leaf blackening.

Period of Packing (days)	Days to Leaf Blackening
1	9 b
2	11 a
3	8 c
4	6 d

* Means in each column followed by the same letter do not differ statistically (P=0.05, Duncan/Waller Multiple range test).

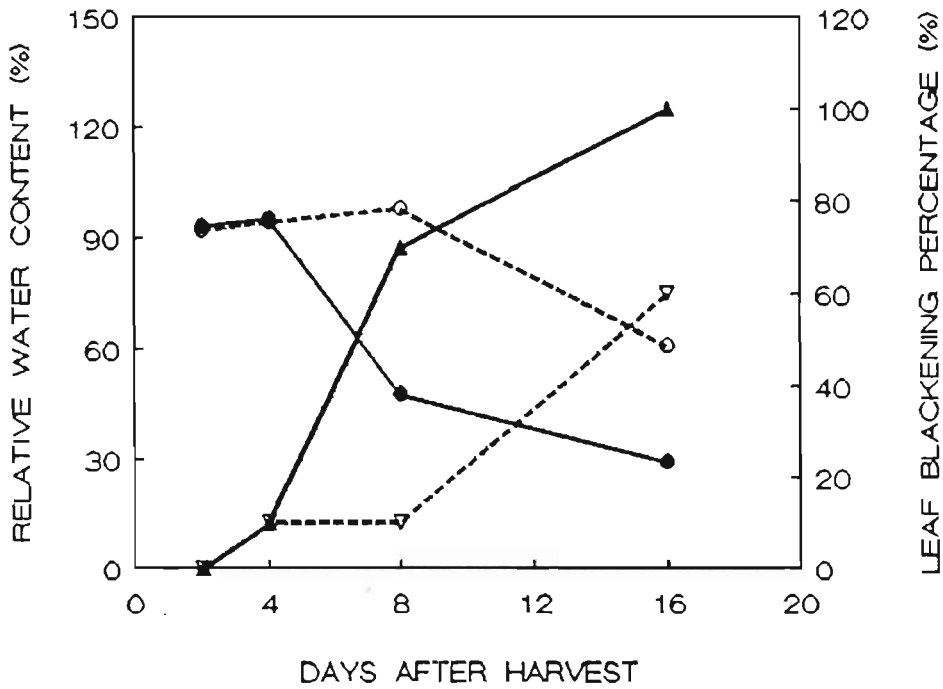

Figure 1 Postharvest changes in leaf relative water content and leaf blackening of *Protea neriifolia* cv Pink Mink. ●——● leaf relative water content of flower held in deionized water, o- - - -o leaf relative water content of flower held in Florever (20 g l^{-1}), ▲——▲ leaf blackening of flowers held in water, and ▽- - - -▽ leaf blackening of flowers held in Florever (20 g l^{-1}).

CARBOHYDRATE STRESS CAUSES LEAF BLACKENING IN PROTEAS

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Abstract

Leaf blackening in cut flowers of *Protea eximia* was not retarded by modifying the vase solution to ensure adequate water uptake (addition of a biocide), by preventing ethylene action (pulsing with STS), by preventing oxidation of phenolics in the vase solution (use of bisulfite), or by reducing transpiration (covering the cut flowers with polyethylene bags). At moderate concentrations, addition of sucrose to the vase solution substantially delayed the onset of leaf blackening, but higher concentrations accelerated the appearance of the disorder. The symptoms were more rapidly expressed when the flowers were held in the dark at room temperature. Removal of the inflorescence, placing the flowers in high light conditions, or girdling the stem immediately below the inflorescence delayed or eliminated leaf blackening. These data suggest that competition for carbohydrate, rather than water stress, is a major cause of leaf blackening in cut stems of *Protea* species. Leaves on cut stems of *P. cynaroides, P. repens, P. punctata,* and *Leucodendron* sp. similarly blackened much more quickly in the dark than in high-light conditions, indicating that the leaf blackening disorder is a common response of proteaceous plants to carbohydrate stress. Practical measures for reducing the occurrence of leaf blackening in harvested proteas are discussed.

1. Introduction

An important postharvest problem with certain species of *Protea,* particularly *P. neriifolia* and *P. eximia,* is premature blackening of the foliage (Ferreira, 1983). Workers in South Africa and Hawaii have studied the nature of the blackening, which results from polymerization of phenols and tannins (Whitehead and de Swardt, 1982), and its causes (Paull et al., 1980). These workers suggest that water stress is a primary cause of the disorder. An intriguing aspect of the disorder is that it can be eliminated by removal of the flower head (Paull et al., 1980), a response which was suggested to be largely due to the reduced water demand of the headless branches. In many flowers, however, water loss from the flowers or inflorescence makes only a minor contribution to overall water loss (Halevy and Mayak, 1979). Because flowers have high rates of respiration and growth, they are major sinks for carbohydrate. We recently reported data which are consistent with a role for carbohydrate stress in the development of leaf blackening in proteas (Reid et al., 1989). In the present study we examine this hypothesis further, and extend our findings to a wider range of proteaceous plants.

2. Materials and Methods

2.1. *Plant material*

Flowers of *Protea eximia, P. cynaroides, P. repens, P. punctata, Leucospermum cordifolium* and a *Leucodendron* sp. were harvested from mature plantings in the Santa Barbara area of California. The flowers were harvested at normal commercial maturity, and either used immediately in experiments in Santa Barbara, or cooled and transported to Davis, and used within 24 hours of harvest.

2.2. *Vase-life evaluation*

Harvested flowers were normally placed individually in water containing 50 ppm hypochlorite, and their vase life tested in standard vase-life conditions (20°C, 60% R.H., 12 hr cool-white light, 18 μmol PAR each day). In some treatments flowers were placed in the dark at 20°C, in a sunny window, or in a greenhouse where the temperature was maintained at 20°C during the day and 18°C at night. Flowers in the greenhouse were placed under 1 layer of cheesecloth (20% shade). In some treatments flowers were girdled, by removing a 5 mm strip of cortex and phloem just below the inflorescence, to restrict movement of carbohydrate between the inflorescence and the leaves.

The effect of the different treatments on leaf blackening was monitored by recording the percentage of the leaf surface that had blackened, or by counting leaves that were more than 10% black.

3. Results

3.1. *Effects of different manipulations on development of leaf blackening*

Leaf blackening in cut flowers of *P. eximia* was not significantly delayed by covering them with plastic bags to reduce water loss (Table 1), but was accelerated when the heads were placed in the dark. Removal of the heads, girdling the stem just below the heads, or holding the flowers in high light substantially delayed the appearance of blackening symptoms, whereas holding the flowers in complete darkness accelerated it.

3.2. *Effects of STS pretreatment and vase solution additives*

Leaf blackening in flowers of *P. eximia* was not affected by pretreating the flowers with 4 mM STS, by adding 50 ppm hypochlorite to the vase solution, or by including 1 mM sodium bisulfite in the vase solution (Table 2) in an attempt to reduce the oxidation of polyphenols which darken the vase water in stems whose leaves are blackening. In contrast, addition of 0.5% sucrose (plus hypochlorite) considerably delayed the appearance of leaf blackening (Table 2).

Table 1 - Effect of different postharvest manipulations on leaf blackening in *Protea eximia*. Mean days to 50% blackening of the leaves. The experiment was concluded after 16 days. Values with no subscript letter in common are significantly different (P=0.01).

Treatment	Vase life
Control	9.4BC
Polybag covered	10.4B
Head removed	16.0A
Girdled	16.0A
High light	15.0A
Dark	7.3C

Table 2 - Effect of chemical pre-treatment and vase solution additives on development of leaf blackening in *P. eximia*. Mean days to 50% leaf blackening. Means with no subscript letter in common are significantly different (P =.01).

Treatment	Vase life
Control	9.4BC
4 mM STS pre-treatment	8.2BC
50 ppm hypochlorite	8.3BC
1 mM NaHSO3	7.2C
0.5% sucrose	16.0A

3.3. *Effect of sucrose concentration*

The development of leaf blackening in leaves of *P. eximia* was markedly delayed by including sucrose in the vase solution (Figure 1). At 1% and higher concentrations of sugar, the quality of the flowers was marred by the development of black spots on the leaves, which were less than the 50% scored in the experiment, but were nevertheless unsightly.

3.4. *Effect of light regime on blackening in different cut protea flowers*

Loss in leaf quality of all the protea species tested was much more rapid when they were held in the dark at 20°C than when they were placed in the greenhouse (data not shown). Quality loss was intermediate in the relatively low-light vase-life evaluation conditions. Leaves of *P. cynaroides* and *P. repens*

never blackened on the flowers held in the greenhouse. Flowers and leaves of the *Leucodendron* species tested were both affected by the light conditions in the postharvest environment. Although leaves of cut flowers of *Leucospermum cordifolium* did not blacken under any of the environmental conditions tested, they did dry out in the low light regimes.

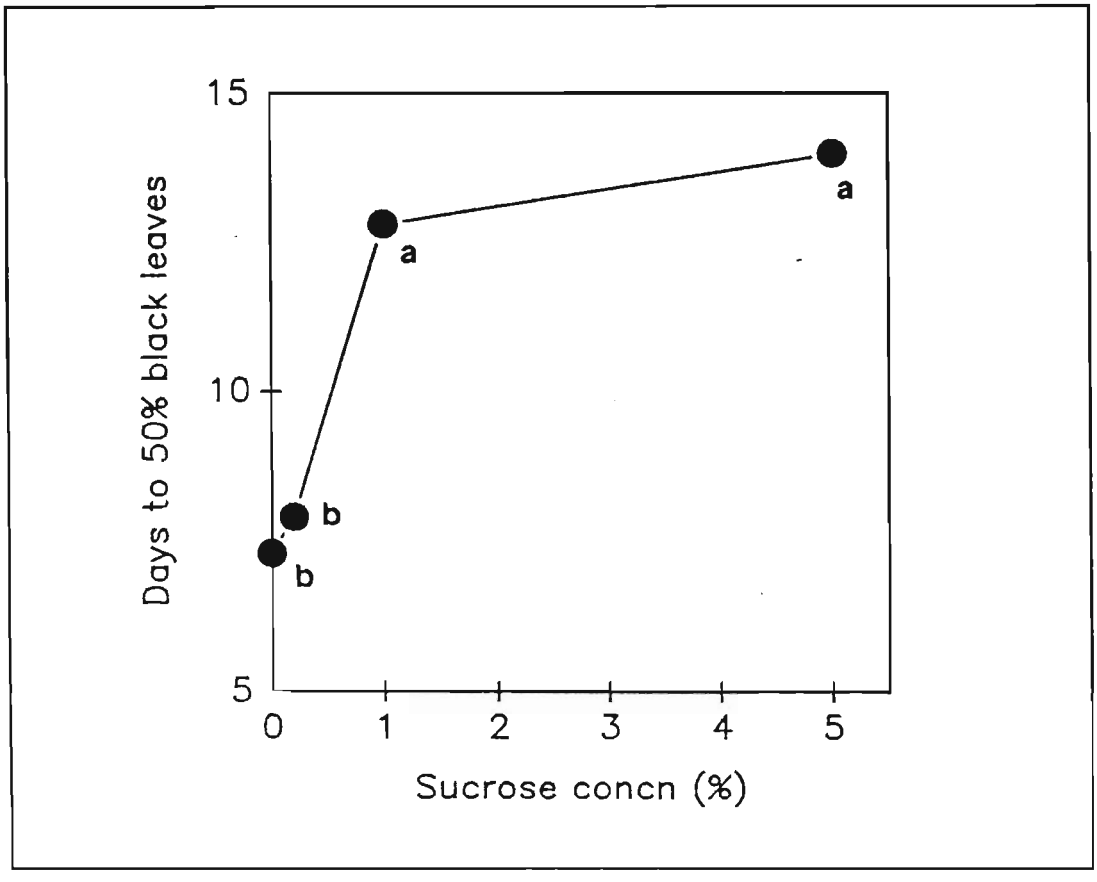

Figure 1 - Effect of different concentrations of sucrose in the vase solution on the time to 50% blackening of leaves on cut inflorescences of *P. eximia*. All vase solutions contained 50 ppm hypochlorite as biocide. Data represent the means of 10 replications. Data points with no letter in common are significantly different ($p=0.1$).

4. Discussion

Leaf blackening is a common symptom of stress in *Protea* leaves, occurring in response to insect or pathogen attack and to severe desiccation. The data reported here show that postharvest blackening of leaves of cut protea inflorescences is not delayed by using hypochlorite in vase solutions which has been reported to improve water relations in these flowers (Faragher, pers. comm.). The fact that drastically reducing water loss by covering the cut

flowers with polyethylene bags did not alleviate the blackening problem suggested another cause for this disorder under normal vase life conditions.

Other workers (Paull, 1980) had reported that removal of the inflorescence reduced blackening, and attributed this response to reduced water loss. In our experiments, the reduction of water loss by the removal of the inflorescence was only modest (data not shown), despite its dramatic effect on leaf blackening. Because the inflorescence comprises many rapidly developing flowers, it seems possible that it is a strong sink for carbohydrate; its removal would therefore greatly reduce carbohydrate demands on the leaves. Furthermore, it is known that the inflorescence has a high respiration rate (Ferreira, 1986). We reasoned that the blackening might be a response to excessive demands for carbohydrates, and our further experiments tested this hypothesis.

Girdling the flower just below the inflorescence, which had no effect on water use, but presumably completely disrupted sugar transport between the leaves and the inflorescence, also greatly reduced the incidence of leaf blackening. This finding strongly supports the hypothesis that the blackening is the result of carbohydrate demand by the inflorescence. There remains, however, the alternative possibility that a phloem translocated toxin from the inflorescence causes blackening of the leaves. If this were the case, it seems unlikely that blackening would be delayed by adding low concentrations of sugar to the vase solution (Table 1). The importance of carbohydrate balance to leaf blackening was further tested by placing flowers under different light regimes. Under dark conditions, blackening occurred more rapidly than in control flowers held in the moderate light conditions of the vase life room. Flowers placed in the stressful conditions of the greenhouse (where they used much more water than in the vase life room) suffered very little from leaf blackening. In the greenhouse, presumably, photosynthesis supplies sufficient carbohydrate to meet the demands of the inflorescence. These data also indicate that carbohydrate demands from the inflorescence are responsible for the blackening of leaves of susceptible *Protea* species under normal vase life conditions.

Our data suggest that vase solutions containing carbohydrate might be used practically to prevent leaf blackening in proteas. Although sugar-containing vase solutions sometimes do alleviate the disorder (Brink and de Swardt, 1986; Fig 1), it is apparent that proteas are also sensitive to sugar. Concentrations higher than 0.5-1% may result in accelerated leaf blackening. Such findings perhaps explain the conclusion of other workers that the leaf blackening is the result of water stress rather than carbohydrate demand.

The practical implications of our findings for producers of proteas that are sensitive to leaf blackening are several. Firstly, it is clear that almost all proteas are sensitive, to some extent, to the carbohydrate stress induced by holding the cut flowers in warm and low light conditions. Flowers should be cooled as rapidly as possible after harvest to reduce respiration and development of the inflorescence. In retail and domestic display, these species

should be held in high light conditions, if possible. The fact that blackening is substantially accelerated in the dark indicates a means by which growers or breeders could select cultivars of species such as *P. eximia* and *P. neriifolia* that are less affected by the problem. Many seedlings could easily and rapidly be screened by placing their cut flowers in a warm, dark environment for a few days. Lastly, the possibility that leaf blackening could be delayed by harvesting flowers in the afternoon, when their carbohydrate status is greatest, warrants examination.

5. References

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6. Acknowlegment

We gratefully acknowledge the support of Dick LaRue, who provided all the flowers used in this study.

POST-HARVEST WATER RELATIONS OF LEUCADENDRON CV. SILVAN RED

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Abstract

Vase life of mature stems of Leucadendron cv. Silvan Red (Ld. laureolum x Ld. salignum) fell from six to four and a half weeks, after exposure to a transient water stress, in the form of strong drying conditions for 30 hr, during which leaf water potentials fell to -3200 kPa.

Vase life was identified by the appearance of localised desiccation of leaves, starting from the tips and extending towards the leaf-base. Ion leakage from the leaves, as a whole, was not detected, but localised leakage of solutes within leaves may have occurred and not been detected. Leaf water relations, were similar for both treatments, with turgor declining from 1300 kPa, initially, to zero by about day 37, and solute potential increasing from -1450 kPa, initially, to -1150 kPa by day 37, indicating loss of solutes. The latter was consistent with loss of dry weight. Transpiration of stressed stems fluctuated in the experiment but fell to a level, similar to the controls and after 45 days had fallen steadily to one fifth of the initial value. Leaf turgor in cv. Silvan Red can be expected to respond to various treatments e.g. pulsing solutions, to improve food supply, but it is not known whether such treatments will alleviate the effects of severe transient stresses on vase life.

1. Introduction

Vase life of cut flowers depends on cell turgor, i.e. plant cell water relations. Yet, measurements of plant water relations are seldom reported in studies of post-harvest treatments designed to prolong vase-life of flowers. In the present study we studied the effect of a water stress on Leucadendron cv. Silvan Red, which is widely grown as a commercial cultivar in Australia; cv. Silvan Red was chosen for its reputedly long vase life.

2. Methods and Materials

2.1. Conditions. Mature stems (80) of cv. Silvan Red were harvested to a length of about 40 cm, held in de-ionised boiled water and taken to the laboratory. Lower parts of stems were then stripped of leaves, recut under water, and put in lots of nine stems per 1 litre plastic beaker, into a weak bromine solution (0.01 g available bromine per litre), for the experiment. To minimise plugging due to micro-organisms, 1 cm of stem was cut off every 5 days and vase solution changed every 2 days.

Stems were exposed to a constant temperature of 20 ^{o}C, and to light from a bank of Sylvania 'GROW-LUX' fluorescent tubes, which provided, over a 12 hr period each day, 150 micro-Einsteins m^{-2} s^{-1} of radiation (measured at half-stem height), which was predominantly in the blue and red bands of the spectrum.

2.2. Water stress treatment. On the day of harvesting, one half the number of stems was taken out of water and exposed to a forced draught of air under continuous light for 30 hr. At the end of this period leaf water potentials varied between -2800 and -3200 kPa. By day 25, no evidence of stress was visible, and so the stress treatment was repeated.

2.3. Measurements. Ion leakage across the plasmalemma was measured on single leaves, 4-8 cm below the involucral bracts, initially every 4 days until day 8, and thereafter every 8 days. The method involved cutting leaves into thin slices, and used a standardized procedure, involving successive rinsings. Finally, the preparation was incubated with gentle agitation for 130 minutes, rinsed, and electrical conductivity of the solution measured after 15 minutes with a PT-18 ACTIVON digital conductivity meter. This procedure was designed to confine ion leakage, during measurement, to ions originating from the symplast. This was indicated by a steady state conductivity response, which followed an initial rapid increase in conductivity of the osmoticum, interpreted as resulting from an initial flush of readily diffusing ions from the apoplast. Leakage was expressed as a percentage of the total conductivity, determined as above, on solution left to equilibrate for 48 hr at 20 ^{o}C, after cell disruption by two cycles of snap freezing in liquid N.

Leaf water potential (ψ_l), solute potential (ψ_s) and relative water content (RWC) were determined at 4-7 day intervals, on leaves (6 replicates) taken from 4-8 cm below the involucre, prior to the onset of the light cycle. ψ_l was determined by pressure bomb (Scholander *et al* , (1965), ψ_s, on expressed sap, by thermocouple psychrometer, and RWC by the method of Barrs (1968). Leaf turgor was calculated using the relationship $\psi_l = \psi_s + \psi_t$, where ψ_t is the leaf turgor potential and usually takes positive values; ψ_s, the solute potential is always negative and has the same value, but opposite sign to the osmotic pressure of the cell sap.

Transpiration, absorption of water, and fresh weight were determined from records of weight changes of vase solution and single stems (4 replicates) sealed into a conical flask and exposed to the same conditions as for the main experiment. Leaf diffusion conductances were determined (10 replicates) by porometry at mid-day every 4- 8 days on the abaxial side of leaves 4-8 cm below the involucre. Bracts on treatment stems exhibited signs of desiccation after the second stress cycle and so it was decided to repeat measurements being made on lower leaves on the bract tissue for both treatments on days 37 and 46. Drying rates of initially turgid, detached leaves and bracts (4 replicates) of both treatments, were determined under prevailing light conditions, at 0.5 to 2 hour intervals over a 12 hour period at the beginning and the end of the experiment.

Pressure-volume (PV) curves were determined on leaves (4-6 cm below the involucre) of freshly harvested stems taken through a drying cycle, using the method of Wilson *et al* (1979), excepting that holding times were greater, varying from 20 minutes, for pressures <1500 kPa, to 30 minutes for pressures >1500 kPa. Leaves were wrapped in plastic film and left overnight in water to become fully turgid. Only leaves with an initial balance pressure of <100 kPa were used and their fresh weight taken as the fully turgid weight for RWC determinations. The inverse balance pressure was plotted against corresponding RWC and the data from 4 replicates pooled for curve fitting. Wilting point was estimated as the negative of the balance pressure at the intersection of curves fitted to the linear and curvilinear parts of the plot.

3. Results

At day 27 no physical differences in the stems could be detected between treatments. By day 15 up to 60 per cent of stems in both treatments had initiated two small lateral shoots arising from the below the involucre. By day 46 these had trebled in size.

During the second stress cycle starting at day 27, 15 per cent of stressed stems had symptoms of irreversible desiccation localized at the tips of the bract leaves. Up to 40 per cent of the bracts from each stem were affected; drying progressed from the tip downwards, for a distance of about 5 mm. This condition worsened as the stems aged. By day 46, 77 per cent of the stressed stems showed varying levels of dessication (Table 1). The severity of the desiccation varied from 3-4 bracts affected per stem to all the bracts being totally dry and the involucre dead. By contrast only 8 per cent of the control stems had bracts, which were beginning to dry out. In both treatments dessication was confined to the bract or the cone.

Table 1 - Stem condition at the end of the experiment.

	Number of stems with symptoms of aging	
Observation	Control	Stressed
Cone dead or dying	0	5
Bracts drying	4	15
Bract and lower leaves drying	0	1
Cone and bracts drying	0	7
Totally dry	0	3
Lateral shoots	28	25

Ion leakage stayed essentially constant, for bracts and leaves of the two treatments, throughout the experiment, at approximately 7 per cent of total conductivity.

Stomata stayed open during the light period as indicated by leaf conductances, which were similar for both treatments. Also drying curves for leaves and bracts, under standard drying conditions, were the same after 46 days. These data did not correlate at all with transpiration, which in the control, declined steadily over the 45 day period to one fifth of the initial value. Stressed stems' transpiration rate was initially 40 per cent of the control rising to 100 per cent at day 14 and thereafter declining rapidly over a 10 day period to reach a similar level to the controls. The initial rapid rise in water usage in the stressed stems, was not observed after the second stress cycle.

Absorption of water was essentially the same as transpiration for both treatments throughout the experiment, but this was not reflected in stem fresh weights, which stayed fairly constant at about 100 per cent of initial fresh weight until day 24, but thereafter declined steadily to 94 per cent in both treatments. Weight of dry matter also declined, by about 14 per cent in both treatments, indicating a net loss of water and of solutes.

Leaf water relations, as indicated by water potentials, were similar for both treatments but varied over the experiment (Figure 1). Turgor was estimated by difference between the ψ_l and ψ_s and declined from 1300 kPa, initially, to zero by about day 37. Solute potential increased from -1450 kPa initially to -1150 kPa. The rise in ψ_s is interpreted as a loss of solutes, and is consistent with the decline in dry matter referred to above. Since

the decline in ψ_l was much greater than could be accounted for by a loss of solutes, it represents an increasing water deficit in the leaves. This was probably due to increasing hydraulic resistance, in the stem conducting vessels, which may have accompanied shrinkage of stem tissue as its water content declined. Since ψ_s was determined at the end of the dark period, the evidence of a water deficit also indicates that transpiration was taking place during the dark period, i.e. that the stomata were staying open.

The critical leaf water potential (ψ_{crit}) (Cowan, 1965), i.e. the value of ψ_l when turgor pressure (ψ_t) = 0, at which pronounced wilting occurs, was estimated from the plot of $1/\psi_l$ v. RWC (Figure 2), as approximately -18 kPa. This value relates to fresh unstressed material at the start of the experiment and compares with a ψ_l of approximately -30 kPa, in the stressed material, also at the beginning of the experiment.

4. Discussion

Vase life of c.v. Silvan Red was of the order of 6 weeks. The experiment failed to detect any general loss of membrane integrity as would be indicated by solute leakage from cells. However the imposed water stress reduced vase life by at least 10 days, the end of which was identified by the appearance of localised desiccation of leaves, starting from the tips and extending towards the leaf base. These observations appear to be similar in kind, and probably have a similar origin to the phenomenon of leaf browning commonly observed in *Protea* spp. (eg. Ferreira, 1986). However they are far milder in intensity and this may be due to a number of factors, including differences in the relative sizes of assimilate sinks and sources in the flower head, and leaves and bracts, respectively, as well as in the abundance of specific compounds, eg. phenolics, in the leaves.

The experiment indicates that when bacterial plugging of xylem vessels is not a serious factor limiting the water supply, vase life of cv. Silvan Red will tend to be correlated with a decline in leaf and bract turgor, caused by reduced osmotic pressure of the cell sap. The data also show that a severe transient water stress may still over-ride, and shorten, any slower, longer term decline, and as a result, reduce the vase-life of the stressed stems.

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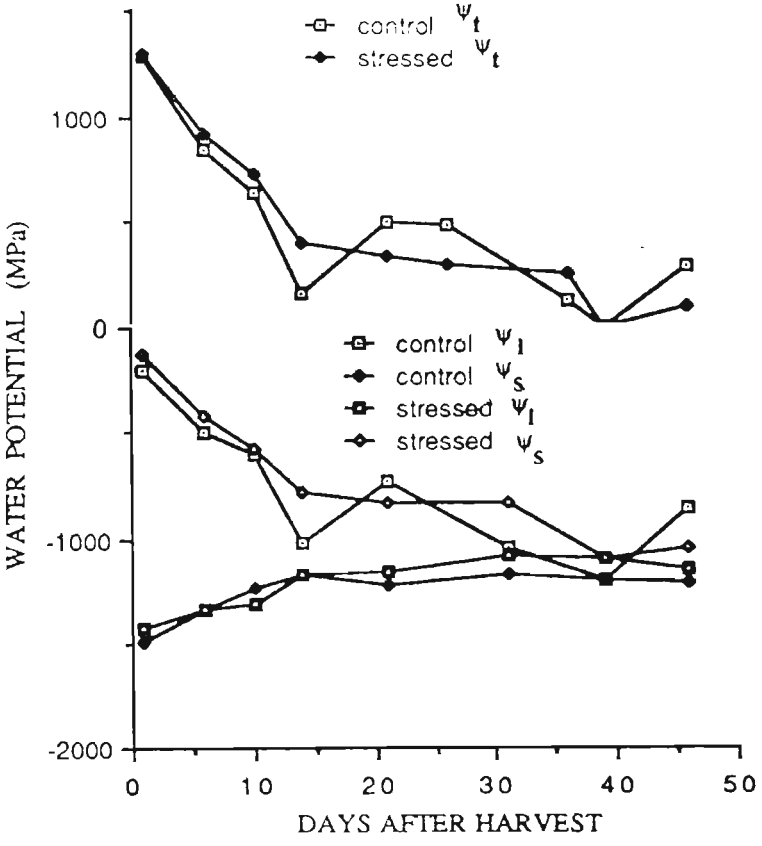

Figure 1 - Changes in leaf water potential (ψ_l) and components, the leaf sap solute potential (ψ_S) and the turgor potential (ψ_t). Each point is the mean value of four leaves from 4 - 6 cm below the involucre.

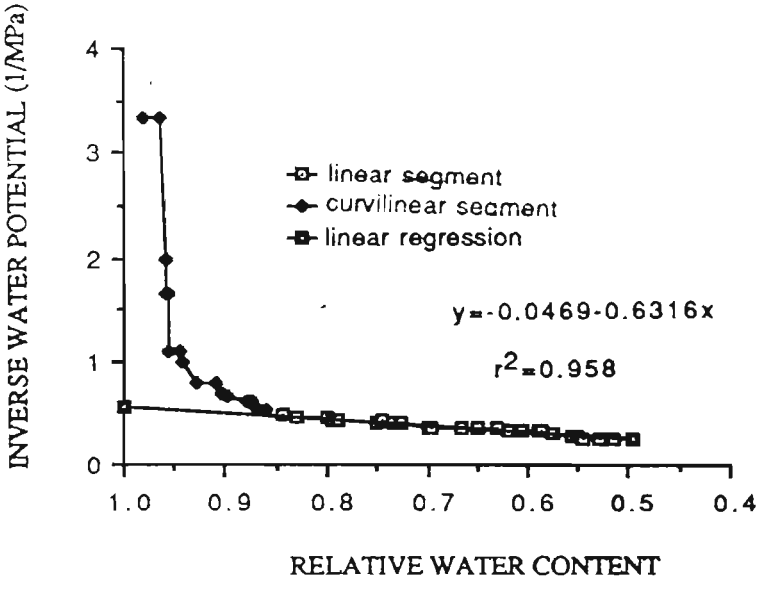

Figure 2 - Pressure-volume curve for Leucadendron cv. Silvan Red leaves (4) taken from 4 -6 cm below the involucre on freshly harvested stems.

EVALUATION OF THE RESISTANCE OF PINCUSHION (*LEUCOSPERMUM* SPP.) BREEDING LINES TO ROOT ROT CAUSED BY *PHYTOPHTHORA CINNAMOMI*

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Abstract

Phytophthora root rot is a limiting factor in the production of cut flower pincushions worldwide, but especially in South Africa. Investigations were initiated to identify root rot resistance within the genus *Leucospermum* that might be utilized for the development of resistant cultivars or rootstocks. Three different methods were used to assess the resistance of *Leucospermum* hybrids and species selections. Firstly, shoot cuttings were wound inoculated with *P. cinnamomi* mycelium under controlled, laboratory conditions. Lengths of lesions which developed from the inoculations were used to estimate relative resistance. This detached shoot assay proved too unreliable to be used for assessment. Secondly, one-year-old rooted cuttings were inoculated by applying zoospores of *Phytophthora cinnamomi* to the soil mix of individual pots in a trial conducted in a shade house. All of the lines tested were found to be susceptible to infection, but some lines were tolerant to infection. Thirdly, field evaluations of resistance were carried out for three years at a site with high natural inoculum levels and other soil factors favoring disease. Good levels of tolerance were observed for several hybrids and species selections. Useful differences in tolerance were also recorded among breeding lines within species. The most tolerant lines are being further evaluated.

1. Introduction

Losses due to Phytophthora root rot are a limiting factor in the production of cut-flowers by pincushions (*Leucospermum* spp.) in major growing regions such as South Africa, Australia and Hawaii (Von Broembsen and Brits, 1985; Von Broembsen and Brits, 1986; Von Broembsen, 1989a). In South Africa, the situation is very difficult because *Phytophthora cinnamomi* occurs on a wide range of indigenous plants in native forests and shrublands (Von Broembsen, 1984a; Von Broembsen and Kruger, 1985; Von Broembsen *et al.*, 1986) and appears to be indigenous to this region (Von Broembsen and Kruger, 1985; Von Broembsen, 1989b). Most pincushions are grown on agricultural lands cleared from or adjacent to native vegetation. They are often irrigated from streams originating in native vegetation and containing high levels of *P. cinnamomi* (Von Broembsen, 1984b). Fungicidal control is difficult with such high inoculum pressure and

lines of *L. conocarpodendron* (lines 3, 1 and 2) grouped together and showed moderate to highly susceptible reactions. However, one of the three *L. cordifolium* lines (line 8) was considerably more resistant than the other two lines (lines 18 and 9). A hybrid between *L. conocarpodendron* and *L. cuneiforme* (line 5) was more resistant than lines of the parent spp. (lines 17 and lines 3, 1 and 2) alone.

4. Discussion

Although Dixon *et al.* (1984) found that a wound inoculation method satisfactorily discriminated between levels of tolerance for *Banksia* spp., the method evaluated here did not give useful results. This may indicate that the range of tolerance within *Leucospermum* is narrower or that the technique they used is more sensitive. Also, the shoots of the *Leucospermum* lines tested varied considerably in diameter and bark thickness, and this may have confounded the lesion length data.

The pot trial demonstrated that all the lines were susceptible using high inoculum levels and conditions that favored infection, i. e. flooding and optimum day/ night temperatures. Certain lines could clearly be identified as being highly susceptible to both infection and disease development. This method could therefore be used to eliminate the more susceptible candidates before taking these into the field for testing. However, some of the lines displaying intermediate tolerance under the artificial conditions of the pot trial demonstrated better levels of tolerance under natural field conditions.

The field trial results showed that good levels of tolerance to Phytophthora root rot are available. Some of the tolerance identified was in lines that could be used directly as cultivars, but most of the tolerance would require further breeding to incorporate it into horticulturally acceptable cultivars. In addition, several of the most tolerant lines are good candidates as rootstocks (Brits, unpublished data). The differences in resistance detected among *L. cordifolium* lines indicate that variation within a species will have to be evaluated carefully. There is also some indication that hybrids may have increased tolerance compared with their parent spp.

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Abstract

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1. Introduction

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with the marked susceptibility of the pincushion varieties currently being cultivated.

Identification of resistance which could be incorporated into cultivars or rootstocks would clearly be an important step towards improved control of this disease. Although the most commonly cultivated pincushions appear to be highly susceptible to Phytophthora root rot, some of the less cultivated species have shown some field resistance. Furthermore, little attention has been given to the possible resistance of species which are not horticulturally suitable without further breeding and selection.

In an earlier pot inoculation study (Von Broembsen and Brits, 1985), five *Leucospermum* spp. which were not considered commercially important showed some resistance. However, only one genetic source of each of these species was evaluated in this experiment. More recently, differences in field susceptibility have been observed among vegetatively propagated lines from the same species in breeding trials, suggesting that variation in resistance to root rot might occur within species.

A program was therefore initiated to identify and evaluate the resistance to Phytophthora root rot available within the genus *Leucospermum*. Breeding lines were selected from the pincushion breeding program of the Vegetable and Ornamental Plant Research Institute for evaluation in a field trial. These included lines from sources which apparently exhibited some resistance in the field as well as a known highly susceptible *L. patersonii* line. Two methods utilizing artificial inoculation of either detached shoots or rooted cuttings were also tested as possible methods of screening larger volumes of material more rapidly than field trials.

2. Materials and methods

2.1 Detached shoot assay

Six *Leucospermum* spp. (table 1) were evaluated using a detached shoot assay in which shoot cuttings were wound inoculated with mycelium of *P. cinnamomi*. The assay method was similar to that reported by Jeffers *et al.* (1981) except that ten cuttings 20 cm in length were used in each beaker and the PCMAA medium was inoculated with *P. cinnamomi* isolate C11. Each ten shoot treatment block was replicated twice. After 10 days, the bark was removed and the lesions which had developed were measured using calipers. The experiment was repeated three times.

2.2 Pot trial

Zoospore inoculum of *P. cinnamomi* isolate C11 was produced axenically using the method of Chen and Zentmyer (1970). Ten ml of an aqueous suspension containing approximately 100 zoospores per ml (1000 zoospores total) was applied to the flooded soil of each two-

liter pot containing a one-year-old rooted cutting of one of the 14 lines being evaluated (table 2). Twenty replicates of each line were inoculated on 5 December 1983. Control plants were not inoculated, but were otherwise treated similarly. All plants were held in a shade house at ambient weather conditions during summer at Stellenbosch, Cape Province and watered daily. Dead plants were removed weekly and isolations were made from their roots to recover *P. cinnamomi* using previously described methods (von Broembsen, 1984a). The trial was terminated at the end of sixteen weeks when the roots of all the surviving plants in the trial were tested for the pathogen.

2.3 Field trial

Eighteen vegetatively propagated breeding lines (table 2) were transplanted during spring (September) 1983 to a site at the Tygerhoek Experimental Farm which had a previous history of severe losses of pincushions to Phytophthora root rot. Single plants of each line were distributed randomly within each of sixty blocks. Dead plants were removed weekly and their roots were tested for *P. cinnamomi* as described above. The trial was terminated after three years.

3. Results

3.1 Detached shoot assay

The results from one detached shoot experiment (table 1) showed that the variability within and between replicates did not allow discrimination between levels of resistance except in extreme cases, such as shown with *L. reflexum*. The data from the other two experiments was even less significant and therefore is not reproduced.

3.2 Pot trial

All fourteen lines were found to be susceptible to infection using this artificial inoculation technique. However, lines differed in their ability to tolerate infection (figure 1) as shown by the time required for each line to reach 50% mortality (LT_{50}). Four lines (16, 10, 13 and 14) were very intolerant to infection. However, four other lines (1, 3, 17 and 18) had not reach the LT_{50} level by sixteen weeks after inoculation when the experiment was terminated. The remaining lines showed intermediate tolerance.

3.3 Field trial

The *P. patersonii* line (line 16) which was included as a susceptible marker was the most susceptible of all the lines (figure 2). In all, six of the eighteen lines in the field trial proved to be highly susceptible (lines 16, 14, 13, 10, 15 and 2). The other twelve lines showed good to moderate field resistance. The three

lines of *L. conocarpodendron* (lines 3, 1 and 2) grouped together and showed moderate to highly susceptible reactions. However, one of the three *L. cordifolium* lines (line 8) was considerably more resistant than the other two lines (lines 18 and 9). A hybrid between *L. conocarpodendron* and *L. cuneiforme* (line 5) was more resistant than lines of the parent spp. (lines 17 and lines 3, 1 and 2) alone.

4. Discussion

Although Dixon *et al.* (1984) found that a wound inoculation method satisfactorily discriminated between levels of tolerance for *Banksia* spp., the method evaluated here did not give useful results. This may indicate that the range of tolerance within *Leucospermum* is narrower or that the technique they used is more sensitive. Also, the shoots of the *Leucospermum* lines tested varied considerably in diameter and bark thickness, and this may have confounded the lesion length data.

The pot trial demonstrated that all the lines were susceptible using high inoculum levels and conditions that favored infection, i. e. flooding and optimum day/ night temperatures. Certain lines could clearly be identified as being highly susceptible to both infection and disease development. This method could therefore be used to eliminate the more susceptible candidates before taking these into the field for testing. However, some of the lines displaying intermediate tolerance under the artificial conditions of the pot trial demonstrated better levels of tolerance under natural field conditions.

The field trial results showed that good levels of tolerance to Phytophthora root rot are available. Some of the tolerance identified was in lines that could be used directly as cultivars, but most of the tolerance would require further breeding to incorporate it into horticulturally acceptable cultivars. In addition, several of the most tolerant lines are good candidates as rootstocks (Brits, unpublished data). The differences in resistance detected among *L. cordifolium* lines indicate that variation within a species will have to be evaluated carefully. There is also some indication that hybrids may have increased tolerance compared with their parent spp.

5. Acknowledgements

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Table 1 - Detached shoot inoculation data.

Leucospermum spp.	Avg lesion lenghth (cm)	SE
L. erubescens	7.65	1.27
L. patersonii	7.35	2.32
L. formosum X tottum	7.10	2.61
L. cordifolium	5.30	2.66
L. utriculosum	5.70	1.54
L. reflexum	1.35	1.18

Table 2 - *Leucospermum* breeding lines evaluated in the pot inoculation trial and in the field trial. Lines 2, 6, 12 and 15 were not included in the pot trial.

1.	150/76/1	*L. conocarpodendron* - Llundudno
2.	150/77/2	*L. conocarpodendron* - Durbanville
3.	151/73/1	*L. conocarpodendron* ssp. *viridum*
4.	T75/11/02	*L. tottum X formosum*
5.	T75/11/24	*L. conocarpodendron X cuneiforme*
6.	T80/06/04	*L. tottum X vestitum*
7.	187/73/1	*L. tottum*
8.	153/77/4	*L. cordifolium* - Houhoek
9.	153/78/1	*L. cordifolium* - Highlands
10.	175/77/1	*L. pluridens*
11.	155/74/1	*L. erubescens*
12.	180/73/1	*L. reflexum*
13.	192/77/4	*L. vestitum*
14.	T80/06/03	*L. vestitum X tottum*
15.	161/77/1	*L. grandiflorum*
16.	173/78/1	*L. patersonii*
17.	T75/11/29	*L. cuneiforme*
18.	'GOLD DUST'	*L. cordifolium* cv 'Gold Dust'

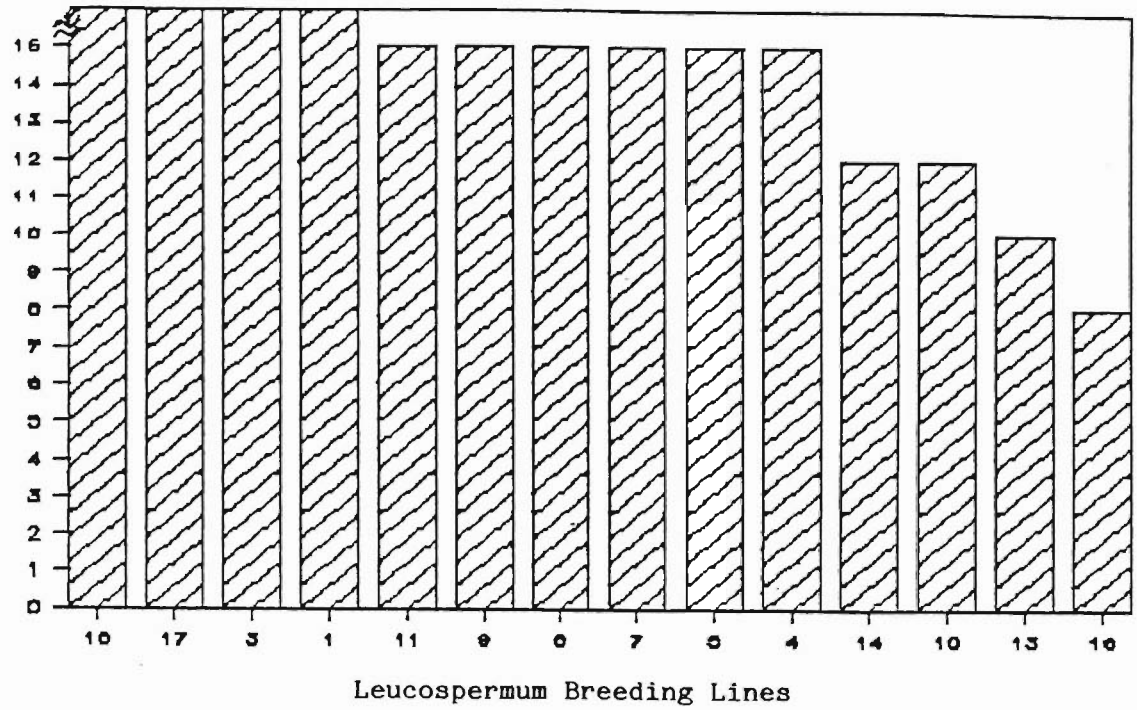

Figure 1 - Number of weeks required to reach 50% mortality due to infection by *Phytophthora cinnamomi* of fourteen *Leucospermum* breeding lines.

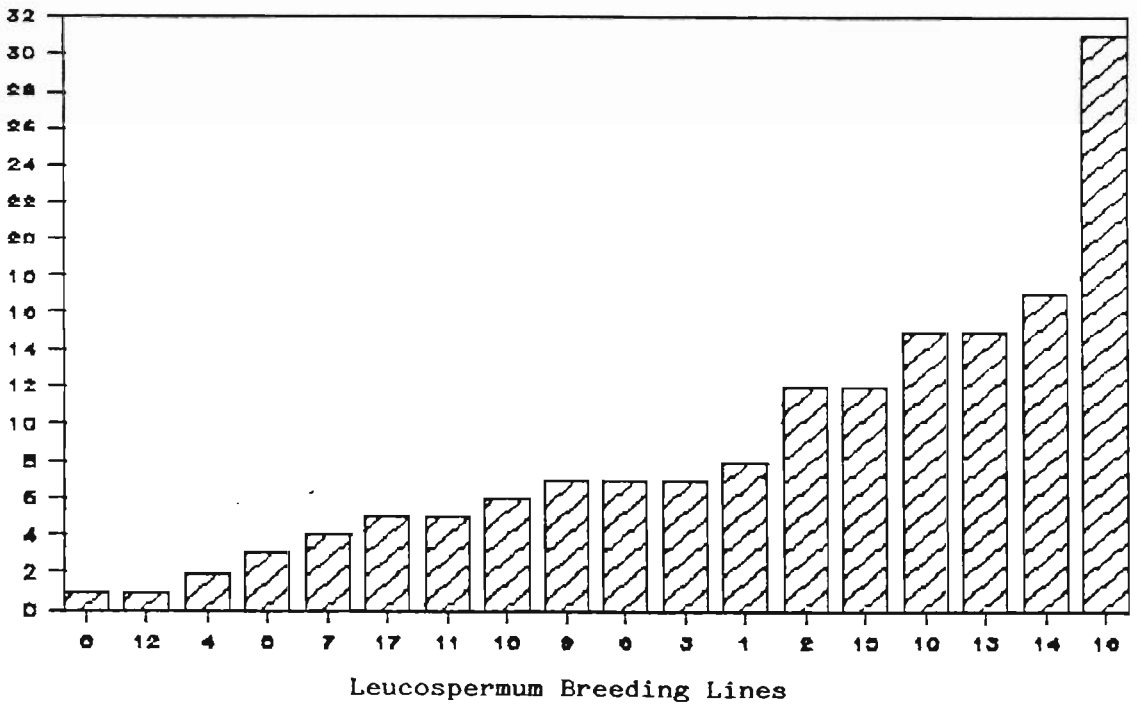

Figure 2 - Number of deaths due to *Phytophthora cinnamomi* out of sixty plants of each of eighteen *Leucospermum* breeding lines after three years (1983-1985).

THE EFFECT OF AMENDMENT OF SOIL WITH ORGANIC MATTER, A HERBICIDE AND A FUNGICIDE ON THE MORTALITY OF SEEDLINGS OF TWO SPECIES OF BANKSIA INOCULATED WITH PHYTOPHTHORA CINNAMOMI

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Summary

This study investigated the benefits of integrated control of the disease caused by the soil borne fungus, *Phytophthora cinnamomi* on two species of *Banksia*. To date no single method has been found to have universal application for control of *P. cinnamomi* in the family Proteaceae. Experiments were undertaken using the Western Australian species *Banksia attenuata* and *B. occidentalis* grown in sand mixed with two types of organic mulch (composted litter from a natural habitat and from an avocado orchard) a fungicide (prothiocarb) and a herbicide (chlorthal dimethyl) singly and in combination. Results show that the type of organic mulch may affect disease expression in the two host banksias and that combination of organic mulch, herbicide and fungicide were relatively the most successful treatments. Integrated control may therefore hold some promise for the control of *P. cinnamomi* in *Banksia*.

Introduction

Row crops of *Banksia* species and other members of Proteaceae in Western Australia are constantly threatened by *Phytophthora* species (Sivasithamparam, 1985) which may originate from production nurseries (Hardy and Sivasithamparam, 1988) or are resident at the sites used to establish wildflower farms. Members of Proteaceae are frequently sensitive to fungicides much as metalaxyl making it difficult and expensive to use such fungicides on a large scale to disinfect sites or to prevent new or re-infection.

Incorporation of organic matter in the form of mulches have shown considerable promise in reducing root-rot in avocado (Pegg et al., 1982). The investigation reported here was conducted to examine whether an integrated control of *Phytophthora* root rot in banksias could be achieved by combined use of organic matter with a fungicide not phytotoxic to banksias and a herbicide reported to suppress *P. cinnamomi* Rands in nurseries (Kassaby, 1985). Two types of organic mulches, one collected around artificially mulched avocado trees and the other from litter collected from natural vegetation at Kings Park at Perth were used.

Materials and methods

Experimental design

An experiment was carried out in a glasshouse, on two species of *Banksia*, using a split plot experimental design. Two types of organic matter amendments formed the main treatments and there were three sub

treatments of a herbicide, a fungicide and herbicide plus fungicide.

Banksia species

Banksia attenuata R.Br. and *B. occidentalis* R.Br. were used as bioassay plants. The former is known to be moderately susceptible to *P. cinnamomi* Rands while the latter is highly susceptible (McCredie, Dixon and Sivasithamparam, 1985).

Organic matter

Two types of organic mulches were used. One was mulched collected from beneth avocado trees at an orchard at Wanneroo, W.A. This was a decomposed mixture of oaten hay and chichen manure. Such mulches are commonly used by avocado growers in Western Australia. The other mulch consisted of litter of native vegetation from Kings Park, Perth.

Both types of organic matter were ground and passed through a 5 mm mesh sieve to obtain an amendment of uniform size. The avocado plantation organic matter was mixed with white organic-free siliceous sand at a ratio of 1:3 (V:V) while the Kings Park organic matter was mixed with the same sand at a ratio of 1:1 (V:V). A lower level of the avocado plantation organic matter was used because it was anticipated that this organic matter could adversely affect the *Banksia* seedlings due to high levels of phosphorus. Each mixture was placed into ten seed trays containing 48 individual pots. Five trays of each organic matter mixture was sown with one species. Two seeds were sown into each individual pot in the seed tray to ensure good germination. In addition seeds of each species were sown into two separate seed trays, containing only sand, which were to act as controls for the experiment.

Maintenance of seedlings

Following seeding the seed trays were placed on stands in the glasshouse and maintained at 20-25C under natural light. Plants were watered every third day. The first seeds germinated twenty five days after sowing. In cases where both seeds in a seedling pot germinated, the last seedling to germinate was removed. In general, the seeds planted in the Kings Park organic matter germinated more reliably than those in the avocado plantation organic matter.

Chemical treatments

The three chemical treatments used in this experiment were a fungicide, a herbicide and combination of fungicide plus herbicide. The fungicide chosen was prithiocarb (Previcur , Schering Pty Ltd.), while the herbicide used was chlorthal dimethyl (Dacthal W75 , Agchem). There were 100 (one month old) plants of each species of *Banksia* in each organic matter type. Therefore each chemical treatment was applied to a group of 25 plants of each species growing in each type of organic matter, leaving 25 plants without a chemical treatment. The control for each treatment had a minimum of ten seedlings. Remaining seedlings were used as uninoculated controls to see if the chemicals or organic amendments were phytotoxic to the test Banksias.

The herbicide treatment was applied at the time of separating the

plants into treatment groups and approximately one month prior to inoculation with *P. cinnamomi*. Dacthal was used at the recommended rate of 9kg a.i./ha (Kassaby and Hepworth) and was applied earlier than Previcur to encourage stimulation of soil microflora and microfauna (Katan and Eshel, 1973).

Previcur was applied at the recommended rate of 3 $ml.2L^{-1}$ of water m^{-2}. Dacthal was also reapplied at this time and both chemicals were reapplied at 4 week intervals throughout the running of the experiment to counter the effect of leaching.

Inoculum Preparation

The isolate of *P. cinnamomi* used to prepare the inoculum for the experiment was isolated from the base of a dead *B. grandis* tree near Armadale (15 km south east of Perth) on the 14th January 1985.

To produce inoculum of *P. cinnamomi* autoclaved millet seed (*Pennisetum miliaceum* L.) was spread evenly over full petri dish cultures of *P. cinnamomi* on PDA. The plates were incubated for 2 weeks at 25C to allow the fungal mycelia to colonize the seeds.

Viability of the millet seed inoculum was tested by taking a random sample of 10 seeds from the inoculum preparation plates. These were plated onto water agar plates, incubated at 25C and after 24 hours hyphae typical of the pathogen was observed growing uniformly from all millet seeds.

Inoculation procedure

All plants to be inoculated with *P. cinnamomi* were placed on a tray which had been lined with heavy duty plastic sheeting to prevent the spread of *P. cinnamomi* in leachates. Plants were inoculated by placing eight infested millet seeds in four holes around the stem base of each seedling (comparable to levels used by McCredie et al., 1985). The holes were positioned approximately 1 cm from the stem base of the seedling and were about 1 cm deep. After inoculation all plants were watered thoroughly.

Disease rating

Plants were observed daily for symptoms of *P. cinnamomi* infection. Infection was considered to be maximum when the hypocotyl was totally necrotized and blackened. the first plant deaths were recorded ten days after inoculation. The experiment was stopped when the inoculated control plants, in sand had reached 100% mortality.

Re-isolation of pathogen

All dead seedlings were tested for presence of *P. cinnamomi* by plating sections of the hypocotyl onto P 10 VP agar. *P. cinnamomi* was isolated from all dead seedlings in the study.

Results and discussion

The organic mulches were variable in their disease reduction effect on the two hosts; the Kings Park mulch mix reducing the disease in *B. occidentalis* with the avocado mulch mix being effective only on *B. attentuata* (Table 1). A repeat of this test however indicated that the unamended avocado mulch mix was effective in reducing disease in *B.*

occidentalis as well (K.W. Dixon, unpublished). Further work clearly needs to be done to investigate the possible existence of host species-specific suppression in different mulch types. It is possible that rhizosphere effect of the two hosts may differ in different substrates. The field resistance of *Eucalyptus calophylla* Lindley to *P. cinnamomi* (causal organism of die-back disease in jarrah, *E. marginata* Donn ex Smith) is considered to result from the activity of specific rhizosphere organisms (Malajczuk, 1979). This resistance was lost when tests were conducted in sterile soil.

There is a clear indication that in using organic mulches for disease amelioration, there is a need for screening the type of organic matter in relation to the host it is used for.

The disease reduction effect of the fungicide was evident only with *B. occidentalis* growing in the Kings Park mulch mix. This result is not surprising as earlier work (K. Sivasithamparam, unpublished) showed that prothiocrab was not as effective as metalaxyl when used as a soil treatment to control *P. cinnamomi*. This fungicide was included as a treatment as it was the least phytotoxic of the fungicides screened in a preliminary study. It was hoped that the effectiveness of fungicide would be enhanced by the background effect of the organic matter. It is noteworthy that combined with the herbicide the fungicide was effective in reducing mortality of *B. occidentalis* in avocado mulch mix. The failure of the herbicide to provide the level of suppression observed by Kassaby (1985) may be related to the differences in potting mixes and experimental methods used. The combination of the three amendments was effective in 3 out of the 4 organic mulch x host species combinations (Table 1). Integrated control appears to hold some promise as a control strategy for *Phytophthora* root rots of proteaceous plants and requires further investigation.

The final mortality values in Table 1 does not provide a complete picture of the treatment effects. Figures 1-4 indicate that several treatments with similar final mortality levels had noticeably different death progression curves. These differences may be significant in the field where the inoculum levels may be much lower than those used in this investigation.

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Table 1. The effect of amendment of soil with mulches from a natural habitat (Kings Park) and an avocado orchard, Dacthal (a herbicide) and prothiocarb (fungicide) on mortality of *Banksia attenuata* and *B. occidentalis* inoculated with *Phytophthora cinnamomi*

		Chemicals			
		Nil	Prothiocarb	Chlorthal dimethyl	prothiocarb + chlorthal dimethyl
Kings Park	*B. attenuata*[a]	76	60	96	76
	B. occidentalis[a]	52*	60*	84	40*
Avocado orchard	*B. attenuata*	44*	48	56	48*
	B. occidentalis	96	84	92	60*

a 100% mortality of both species in the absence of organic mulch

* significantly less than control ($P<0.05$)

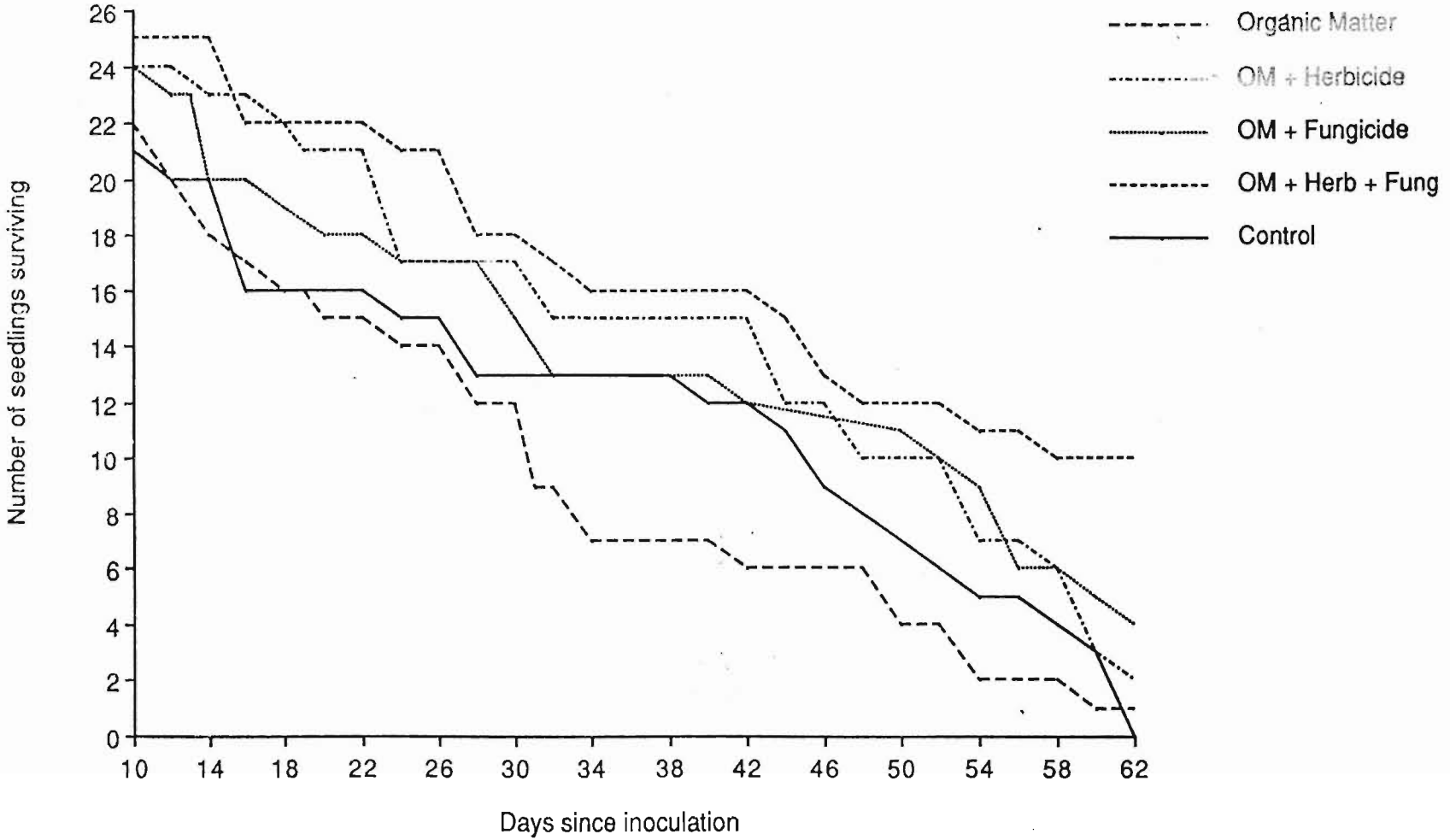

Figure 3 The effect of four treatments on the survival of B. occidentalis growing in avocado plantation organic matter

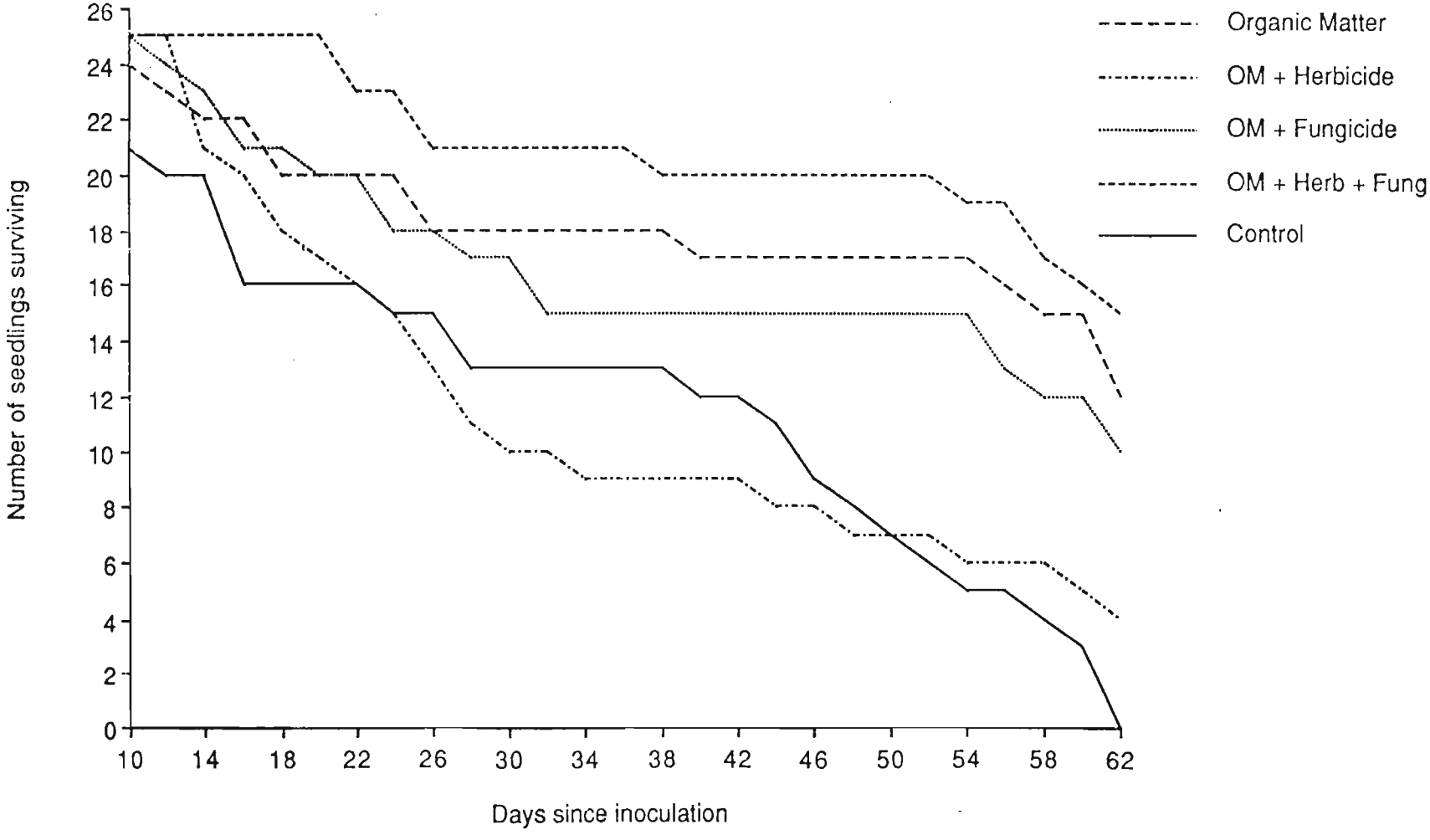

Figure 4 The effect of four treatments on the survival of B. occidentalis growing in Kings Park organic matter

CANKER AND DIE-BACK OF CUT-FLOWER PROTEAS CAUSED BY BOTRYOSPHAERIA DOTHIDEA: EPIDEMIOLOGY AND CONTROL.

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ABSTRACT

Stem canker and die-back caused by Botryosphaeria dothidea result in considerable losses in the prdouction of cut-flower proteas in South Africa. Field and laboratory studies were carried out to investigate conditions favouring the disease and methods of control. The seasonal pattern of B. dothidea sporulation in a naturally infected planting of Protea grandiceps was determined by weekly trapping and counting of conidia for one year. Climatological data such as temperature, rainfall, and relative humidity were recorded throughout the trial. Sporulation occurred from spring to late summer following rain. Sporulation was negligible during winter months despite abundant rainfall. The most significant factors affecting sporulation were average daily temperatures above 20 C and rainfall. The optimum temperature for radial growth of B. dothidea on malt agar plates was 25 C. Conidial production on malt agar and germination in distilled water were greatest at 20-25 C. Infection studies using potted seedlings of P. grandiceps and P. cynaroides in controlled environment cabinettes showed that wounding was necessary for infection and that infection was greatest at 25-30 C.

The effects of three fungicides (benomyl, captab, and mancozeb) and six fungicidal wound sealants on mycelial growth, conidial production and conidial germination by B. dothidea were examined in the laboratory. Benomyl added to media at 1000 ppm completely inhibited all three processes, and Hostaseal gave the best inhibition of the wound sealants. However, benomyl applied as a spray following pruning gave better field control of the infection of pruning wounds than Hostaseal. In a field trial combining several different control strategies, monthly spray applications of the pesticides, chlorophyriphos and dimethoate, in combination with benomyl and captab gave better control than the two fungicides alone. This supports field observations that cankers are often associated with insect wounds. However, this trial also showed that the single most effective control measure was regular removal and destruction of all dead and dying plant parts. Control of Botryosphaeria canker can thus be brought about by preventing unnecessary wounding of plants, by treating harvesting and pruning wounds with a fungicidal spray of benomyl, and by regular sanitation of plantings.