SEED AND SEEDLING ECOLOGY OF FOUR AGULHAS PROTEACEAE

Penelope Jane Mustart

Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Botany
UNIVERSITY OF CAPE TOWN
July, 1991

I dedicate this thesis to my late mother, in tribute
to her love and loyalty.

To God Be the Glory: Without whose creation this study
would not have been, and without whose guidance this
journey would have neither begun, nor finished.

ABSTRACT

SEED AND SEEDLING ECOLOGY OF FOUR AGULHAS PROTEACEAE

July 1991

Penelope Jane Mustart: Botany Department, University of Cape Town, Private Bag, Ronbebosch 7700, South Africa.

Serotinous (canopy-stored seed) Proteaceae, Protea obtusifolia and Leucadendron meridianum occur on shallow, alkaline soils overlying limestone, and P.susannae and L.coniferum on adjacent, deep, weakly acidic sands, in fire-prone fynbos. Seed reciprocal transplants were used to test whether regeneration niche requirements were determinants of adult distributions. There were no germination niche differences, but limestone species showed greater seedling mortality on the transplanted colluvial sands than limestone. There were also greater relative growth rates of seedlings on their own soil than counterparts on transplanted soil.

Mechanisms of field emergence patterns (greater and less variable (in space) emergence of all species on limestone than colluvial sands; less variable emergence in Protea spp. than Leucadendron spp. on colluvial sands) were investigated in laboratory experiments. Seeds planted in limestone soil were more adequately buffered against drying-induced, seed-imbibed water loss, than those in colluvial sand, suggesting the role of soil type in determining germination success. Protea spp. lost less water than Leucadendron spp., suggesting that the different genus-specific seed morphologies influence germination.

Laboratory determined seed germination rates showed that older canopy-stored seed were slower in germinating than current year seed. This was interpreted as a bet-hedging strategy ensuring maximum post-fire germination in dry environments.

Within each genus seeds, and roots of seedlings, were larger in species naturally occurring on the drier, less nutrient rich colluvial sands than in those on the limestone, possibly leading to adequate resource acquisition of the former species. The greater root lengths of the Leucadendron than the Protea spp. balanced the taxonomically linked smaller seed size of the former.

Limestone soils, which had less volume than the deep colluvial sands, supported smaller plants with associated lower cone and seed numbers. There were no other soil-related differences in reproductive traits (except seed size) among the species.

Experimental inflorescence and infructescence harvesting resulted in seed bank depletion of up to 50%. Inflorescence (sink) removal of Protea spp. showed no subsequent increase in seed set in remaining infructescences, negating a nutrient-limiting hypothesis of low seed set.

ACKNOWLEDGEMENTS

I thank my husband, John, for his consistent love and moral support, and for ignoring my cries to "give it all up" at difficult times.

My supervisor, Richard Cowling, I thank for guidance, encouragement and friendship, and for always giving me the confidence to do the task at hand. He has freely shared his knowledge and love of the Agulhas Plain flora. I have benefited greatly from this, as I have from his generosity of spirit, and I will always be grateful.

In most of my field work I was assisted by Loulene Rupert (now Kuschke). Her unfailing good humour and practicality helped make those days working in the field in sun, wind and rain profitable. John and Janice Albertyn of Kleiheuwel, and Ian and Avanol Bell of Hagelkraal, provided hospitality and friendship, the use of their farms, as well as the occasional rescue when my vehicle was stuck in the mud and sand. Their help was invaluable, and I am indebted to them all.

I am grateful to Wendy Paisley for field assistance and backup support in the laboratory. George Davis and Willie Stock willingly gave much practical and theoretical advice.

Helpful discussions were had with W.J.Bond, H. Laurie, G. Midgeley, H. Moolman and R. Scholes. D.Dunne provided statistical advice. W.J. Bond, G.W. Davis, B. Lamont, O.A.M. Lewis, W.D. Stock and E.T.F. Witkowski commented on parts of the thesis.

M. Cocks performed the seed nutrient analyses and D. Barnes gave assistance in the greenhouse experiment. The following helped with the tedious task of seed sorting: E. Azorin, N. Jacobs, M. Johnson and W. Paisley. J. Barker prepared many of the diagrams.

I am grateful for the help and friendliness of so many in the Botany Department. H. Botha, in particular, is thanked for his efficiency in dealing with any practical request. For friendship, comeraderie and help in so many ways, a last, but not least, thank you to all friends in Ecolab 1 & 2.

I am grateful for financial support received from the Fynbos Biome Project of the Foundation for Research Development, and the South African Nature Foundation.

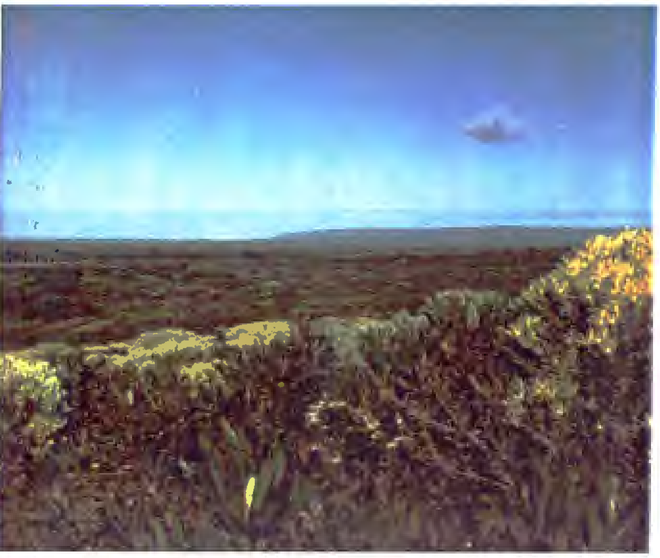
1

2

3

4

5



6

TABLE OF CONTENTS

Section 1

1.0 INTRODUCTION

1.0 INTRODUCTION

1.1 GENERAL BACKGROUND

The Agulhas Plain at the southern tip of Africa is in some places quite bleak, and in others it is breathtakingly beautiful. In similar fashion, the vegetation can be either monotones of cultivated wheatfields, or changing kaleidoscopes of natural fynbos plant assemblages. This high turnover of plants indicates a multitude of plant processes in determining such variety. The plant communities (Cowling et al. 1988) and soil-plant associations (Thwaites & Cowling 1988) have been described and mapped, thus laying the foundation for research at a finer scale.

The flora of the south-western Cape Province of South Africa, of which the Agulhas Plain is a part, is extra-ordinarily rich in species (Taylor 1978, Bond 1983, Bond & Goldblatt 1984). Much of this variety in species is due to high levels of beta (between habitat) and gamma (within landscape) diversity (Kruger & Taylor 1979, Linder 1985, Cowling 1990), a feature shared with other species rich floras such as south-western Australia kwongan (Bond & Goldblatt 1984, Lamont, Hopkins & Hnatiuk 1984), Californian chaparral (Cody 1986 a) and tropical rainforests (Gentry 1988). What causes such high species turnover ? The Agulhas Plain fynbos vegetation has high levels of edaphic endemism (Cowling & Holmes, in press) and soil-related beta-diversity (Cowling 1990). This indicates strong relationships between plant distributions and soil type.

The distinct distribution of two closely-related Agulhas

Plain Proteaceae species-pairs on adjacent, different soil types is a representative cameo of this beta-diversity pattern, and forms the basis of this study. Throughout this thesis there is a constant theme - the role of the soil environment, and that of phylogeny, in determining seed, seedling and adult characteristics, and the subsequent influence in adult distribution patterns. Since the soils are adjacent to each other, the effect of mesoclimate and fire regime would be similar for all species. Since each of the pairs of species is closely related (putative sister taxa), the role of ecological factors in shaping plant traits can be investigated without the constraints of phylogeny (Wanntorp _et al._ 1990).

1.2 STUDY SPECIES AND STUDY AREA

The species studied were _Protea obtusifolia_ Beúk ex Meisn. and _Leucadendron meridianum_ I.Williams, occurring in shallow pockets (0 to 30 cm deep) of alkaline soil derived from Mio-Pliocene limestone bedrock of the Bredasdorp Formation (Thwaites & Cowling 1988). The other species, _P.susannae_ Phill. and _L.coniferum_ (L.) Meisn., occur on the adjacent, uniformly deep (>1 m), weakly acidic colluvial sands. The former soils have higher topsoil nutrient concentrations (nitrogen, available phosphorus and organic carbon) and have a higher proportion of clay than the latter (Thwaites & Cowling 1988). The two _Protea_ spp. (Rourke 1980) and the two _Leucadendron_ spp. (Williams 1972) are both closely related sister taxa.

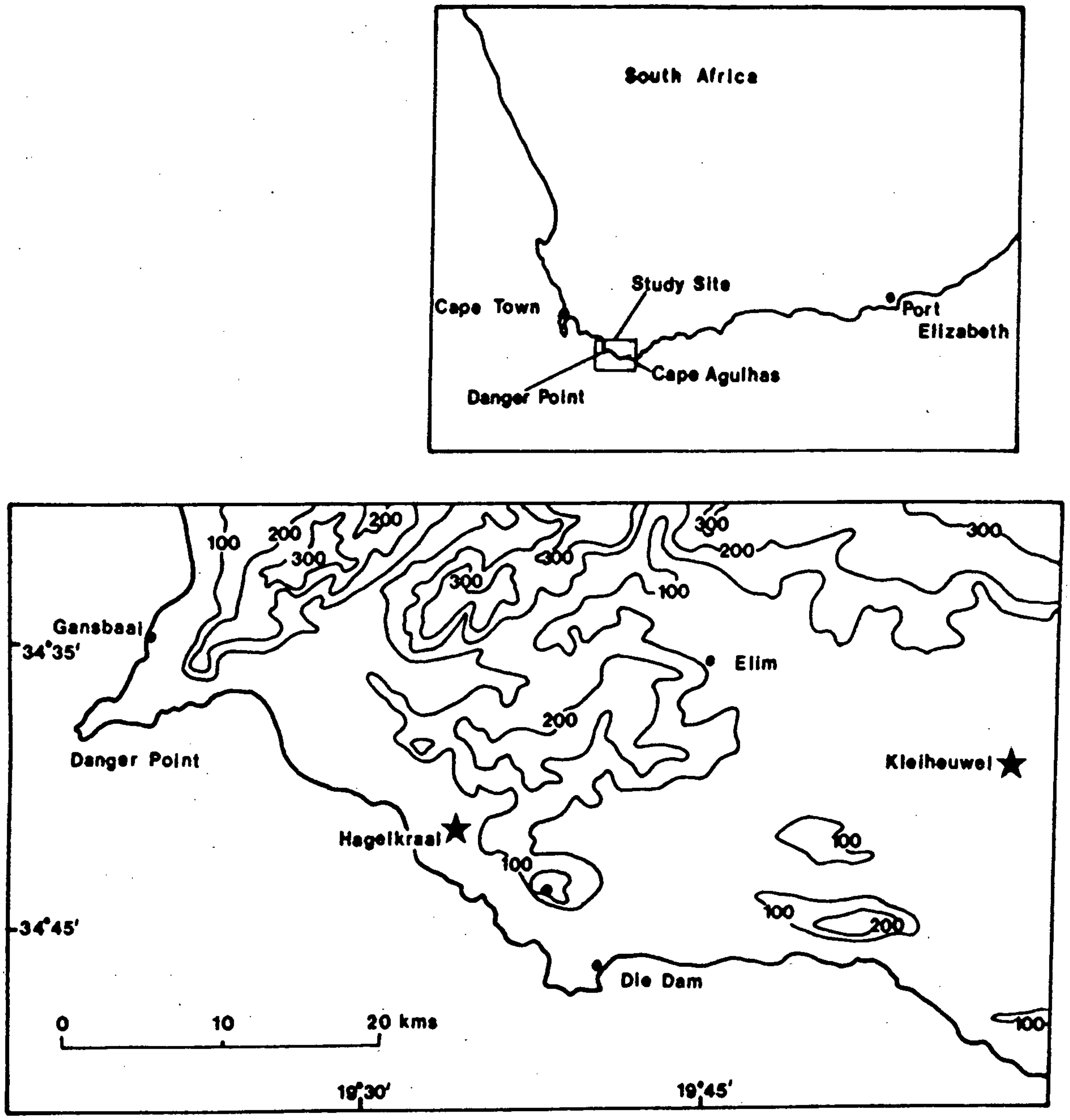


Figure 1.1. Map showing the location of the study sites at Hagelkraal and Kleiheuwel on the Agulhas Plain, South Africa.

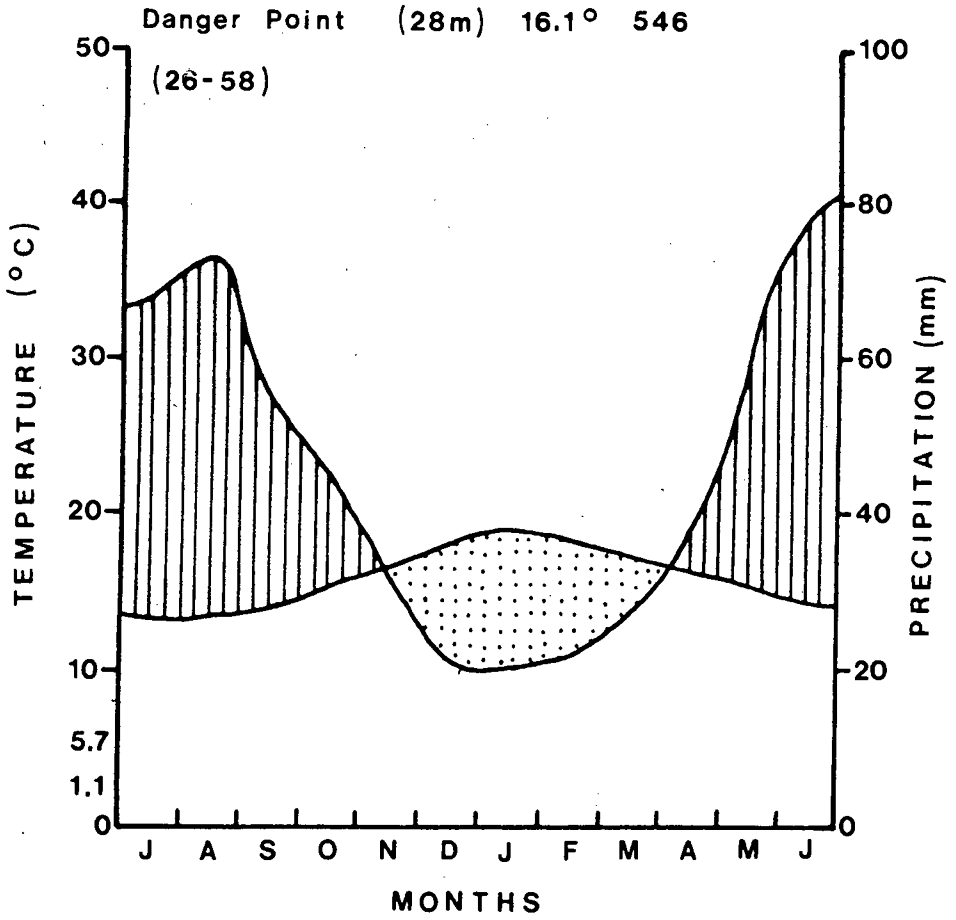
These Proteaceae species are dominant, woody shrubs (1.5 to 3.0 m tall) in the fire-prone Proteoid Fynbos vegetation. The seed are achenes and are stored on the canopy (serotiny) in woody infructescences (hereafter called "cones"). Seed of the hermaphroditic Protea spp. are born on flattish, slightly convex receptacles, and in the dioecious Leucadendron spp. in cones where a woody bract protects each seed. The plants are killed by fire, usually in the dry summer season. This leads to seed release and is followed by large-scale germination during the wet winter season. Seed released between fire do not develop into seedlings, and this leads to even-aged populations.

Two areas were chosen for study sites (Figure 1.1). The Hagelkraal site (34° 41'S, 19° 33'E) was used for seed reciprocal transplants in post-fire soil, five weeks after the mature vegetation had been burnt (Section 2). The 18 year-old vegetation at the second study site at Kleiheuwel was used to investigate adult plant reproductive traits (Section 6), and the effect of experimental harvesting on seedbanks (Section 7).

The climate is mediterranean with 65% of the annual rainfal occurring between April and September. Annual rainfall is 546 mm at Hagelkraal and 452 mm at Kleiheuwel (see Figure 1.2).

1.3 SOIL REGIME IN RELATION TO SEEDS, SEEDLINGS AND ADULTS

At different stages of plant development from seed to adult, the relationship between soil and the plant differs as size relationships between the two change. For example, the limestone soil represents a more nutrient rich micro-environment for seeds and seedlings than the colluvial sands, whereas due to the



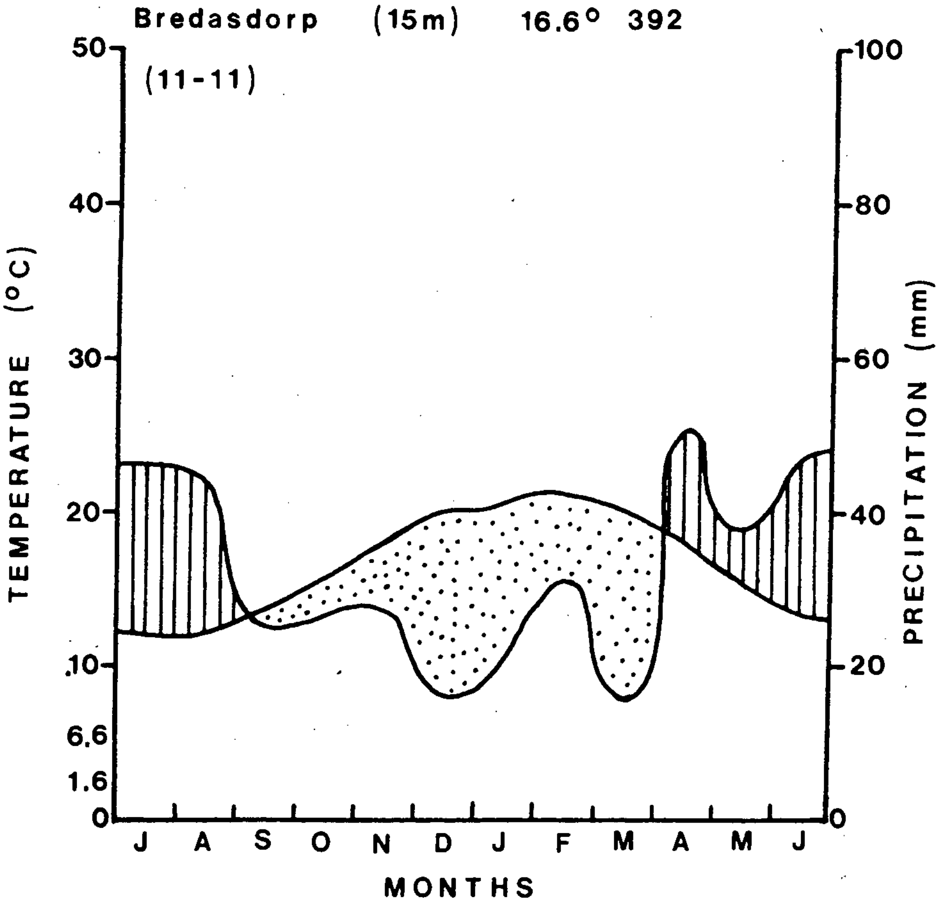


Figure 1.2. Walter-Leith climate diagrams prepared from data collected at Danger Point and Bredasdorp (10 km north-east of Kleiheuwel).

very much smaller soil volume of the former (shallow pockets) than the latter (>1 m deep), the reverse would probably hold for the larger adults.

1.3.1 Soil regime, seeds and seedlings

The importance of investigating seed ecology in order to fully understand adult plant density and distribution patterns is stressed by many authors (Harper, Williams & Sagar 1965, Mayer & Poljakoff-Mayber 1975, Grubb 1977). Seed of serotinous fynbos plants that are killed by fire are the sole link between generations. It follows that it is important to understand the factors affecting seed germination and establishment in order to understand post-fire fynbos recruitment patterns.

Post-fire recruitment patterns of fynbos Proteaceae are usually explained in terms of post-dispersal seed predation levels (Bond 1984) and little work has been done on individual species seed and seedling biology. In addition, numerous studies on South African Proteaceae germination requirements (van Staden 1966, Brown & van Staden 1971, 1973, 1975, Brown & Dix 1985) have been performed with the aim of understanding germination per se and not in order to understand ecological recruitment patterns. I have investigated aspects of seed and seedling ecology in order to explain recruitment and adult population patterns.

I have performed both field and laboratory experiments. In the latter, I have where possible, tried to simulate field conditions, or at least plan and interpret the experiment within an ecological framework. The relevance of laboratory experiments in explaining field processes is questioned by many as bearing little relevance to field processes (Grubb 1977, Norton 1987). Although this may be valid, laboratory experiments are often the only way in which to attempt the unravelling of complex field events. Laboratory experiments perform a function in botanical research as much as, for example, mathematical modelling does. The latter approach, with all its assumptions, and hence restricted explanatory power (Shmida & Ellner 1984, Caswell 1988, Fagerström 1988), is as limiting as laboratory experiments are with their laboratory-imposed conditions. Yet, both approaches are valuable. In this study I have used field and laboratory experiments to complement each other.

What determines the distinct distribution patterns of the study species? Classical niche theory (Gause 1934) predicts that plants have a particular subset of habitat conditions to which they are suited, ar adapted. Grubb (1977) developed the concept that regeneration attributes (or niches) are important in determining adult distribution patterns. In addition to abiotic factors determining plant habitat patterns, biotic inter-plant competitive effects are suggested to be important in determining community structure (Cody 1986 b). The relative importance of abiotic (soil nutrients and moisture) and biotic factors is determining selected Agulhas Proteaceae distributions in currently under investigation (M.D. Richards, in preparation). I have excluded competitive effects, and have investigated the role of the regeneration niche (seed germination and seedling establishment) in order to understand the determinants of the study species characteristic distributions.

The calcicole and calcifuge plant taxa occurring on calcareous and acid soils have been widely studied in Continental Europe (eg. Gigon 1971), England (eg. Grubb, Green & Merrifield 1969, Etherington 1981) and North America (eg. Wentworth 1981). Experimental evidence suggests that seedling stages are determinants of these adult distributions (Jefferies & Willis 1964, Rorison 1967, Grime 1965, Gigon 1971). In order to investigate the role of the regeneration niche in the Agulhas Plain study species distributions, seed reciprocal transplants were performed in the field (Section 2) and seedling emergence and establishment was monitored. The hypothesis that the species have germination and/or seedling requirements that limit them to their respective soil types, is tested.

Temperature requirements for seed germination, and germination rates of different ages of canopy-stored seed were investigated in controlled environment growth chambers (Section 3).

The importance of different levels of water availabilty in determining germination success is widely accepted (Bewley & Black 1978). The emergence patterns linked to microhabitat variability (Harper, Williams & Sagar 1965, Reader & Buck 1986, Hamrick & Lee 1987, Fowler 1988) have been suggested to be caused by small scale differences in water availabilty. Since soil is the basic source of water for plants, and since soils with different textures have different water-retention properties (Karamos 1981), it follows that it is important to relate field seed germination to such soil water properties.

Seedling emergence has been shown experimentally to be linked to the availabilty of water in soils of different particle sizes (Keddy & Constabel 1986). Seed germination is the outcome of water gained and water lost (Mayer & Poljakoff-Mayber 1975). Thus an important factor leading to seed germination is the ability of seed to retain imbibed water in the face of the strong opposing evaporative drying power of the atmosphere. Since seed of serotinous Proteaceae species are released on to the denuded post-fire soils, seed imbibed water retention would be a major determinant of eventual germination. Studies have investigated the ability of seed to imbibe and retain water on artificial substrates (Harper & Benton 1966, Hegarty 1977), but there have been no studies investigating the process of seed - imbibed water retention in relation to the actual soil water properties. I have investigated the relationship of soil type and seed morphology with seed-imbibed water retention in laboratory experiments (Section 4) in order to explain the different emergence patterns found on the different soil types in the reciprocal transplants (Section 2).

The role of environmental factors in determining seed size has been extensively investigated in surveys that correlate habitat characteristics with seed size (Baker 1972, Salisbury 1974, Foster 1986, Mazer 1987). Taxonomic factors have also been shown to account for much seed size variation (Hodgson 1989, Mazer 1987). The idea that soil nutrients and moisture act as a selective force in determining seed size was explored in relation to the phylogenetic constraints of the two species-pairs (Section 5). Laboratory-determined root:shoot ratios and seedling relative growth rates were determined and used to explain how the species of two genera co-exist on each soil type.

In the General Discussion I pull together these aspects of seed and seedling behaviour and I highlight the value of obtaining this autecological knowledge in order to explain post-fire population recruitment patterns (Section 8).

1.3.2 Soil regime and adult plants

The soils that fynbos plants grow in are, in general, nutrient-poor (Taylor 1978). Trade-offs between the different plant functions of vegetative growth, defence and reproduction are reported to occur (Harper 1977, Bazzaz *et al.* 1987, Ronsheim 1988). Since Proteaceae have seeds with highly concentrated nutrients (van Staden & Brown 1977, Pate *et al.* 1985), and since the study species occur on soil habitats with different nutrient concentrations, it can be anticipated that there will be differences in patterns of cone and seed accumulation according to soil type. Selected reproductive traits were quantified and interpreted in this light (Section 6).

1.4. HARVESTING IMPACTS

A wide variety of Proteaceae species are extensively harvested for their commercially valuable cones and inflorescences. The impact of such harvesting on seed banks is not known, and is considered important in order to predict the effect of harvesting on post-fire recruitment (Cowling, Lamont & Pierce

1986, Van Wilgen & Lamb 1985). I investigated this by studying the relationship between cone numbers and canopy volume in harvested and unharvested populations. Controlled harvesting on previously unharvested populations was performed in order to ascertain the effect of such harvesting on future cone production, and, in the two Protea spp., on seed set (Section 7).

1.5 THESIS LAYOUT

The thesis sections were originally prepared for submission to different journals and this has resulted in each having a slightly different formats. Whilst I have attempted to make the thesis more uniform, there will remain some discrepancies. The study site and species description given in this introduction is repeated in each section as is relevant to the particular topic. Thus there exists a measure of repetition in this respect.

In order to obtain a brief overview of the thesis I suggest the following are read: the Introduction (Section 1), abstracts of Sections 2 to 7, and the General Discussion (Section 8). This could be followed by a detailed reading of Sections 2 to 7.

1.6 REFERENCES

Baker, H.G. (1972). Seed weight in relation to environmental conditions in California. Ecology 53, 997-1010.

Bazzaz, F.A., Chiariello, N.R., Coley, P.D. & Pitelka, L.F. (1987). Allocating resources to reproduction and defence. Bioscience 37, 58-67.

Bewley J.D. & Black, M. (1978). Physiology and Biochemistry of Seeds in Relation to Germination. I. Development, Germination and Growth. Springer-Verlag.

Bond, W. (1983). On alpha diversity and the species richness of the Cape Flora: a study in southern Cape Fynbos. From: Kruger, F.J., Mitchell, D.T. & Farvis, J.U.M. (Eds). Ecological Studies, Vol. 43: Mediterranean-Type Ecosystems, Springer-Verlag.

Bond, W. (1984). Fire survival of Cape Proteaceae - influence of fire season and predators. Vegetatio 56, 65-74.

Bond, P. & Goldblatt, P. (1984). Plants of the Cape flora: A Descriptive Catalogue. Journal of South African Botany, Supplementary Volume No. 13.

Brown, N.A.C. & Dix, L. (1985). Germination of fruits of *Leucadendron tinctum*. South African Journal of Botany 51, 448-452.

Brown, N.A.C. & van Staden, J. (1971). Germination inhibitors in aqueous seed extracts of four South African Proteaceae. South African journal of Botany 37, 305-315.

Brown, N.A.C. & van Staden, J. (1973). The effect of scarification, leaching, light, stratification, oxygen and applied hormones on germination of Protea compacta R.Br. and Leucadendron daphnoides Meisn. South African Journal of Botany 39, 185-195.

Brown, N.A.C. & van Staden, J. (1975). Inhibitors are not responsible for poor germination of Protea compacta seed. South African Journal of Botany 41, 225-230.

Caswell, H. (1988). Theory and models in ecology: a different perspective. Ecological Modelling 43, 33-44.

Cody, M.L. (1986 a). Diversity, rarity, and conservation in Mediterranean-climate regions. From: Soule (Ed.), Conservation Biology: the Source of Scarcity and Diversity. Sinauer, Sunderland.

Cody, M.L. (1986 b). Structural niches in plant communities. From: Diamond J. & Case, T.J. (Eds). Community Ecology. Harper and Row, New York.

Cowling, R.M. (1990).Diversity components in a species-rich area of the Cape Floristic Region. Journal of Vegetation Science 1, 699-710.

Cowling, R.M., Campbell,B.M., Mustart, P.J., McDonald, D.M., Jarman, M.L. & Moll, E.J. (1988). Vegetation classification in a floristically complex area: The Agulhas Plain. South African Journal of Botany 54, 290 - 300.

Cowling, R.M. & Holmes, P.M. (in press). Endemism and speciation in a lowland flora from the Cape Floristic Region. Biological Journal of the Linnean Society.

Cowling, R.M., Lamont, B.B. & Pierce, S.M. (1986). Seed bank dynamics of four co-occurring Banksia spp. Journal of Ecology 75, 289-302.

Etherington, J.R. (1981). Limestone heaths in south-west Britain: their soils and the maintenance of their calcicole-calcifuge mixtures. Journal of Ecology 69, 277-294.

Fagerström, T. (1988). Lotteries in communities of sessile organisms. Trends in Ecology and Evolution 3, 303-306.

Foster, S.A. (1986). On the adaptive value of large seeds for tropical moist forest trees: a review and synthesis. The Botanical Review 52, 260-299.

Fowler, N.L. (1988). What is a safe site?: Neighbour, litter, germination date, and patch effects. Ecology 69, 947-961.

Gause, G.F. (1934). The struggle for Existence. Williams & Wilkins, Baltimore.

Gentry, A.H. (1988). Changes in plant community diversity and floristic composition on environmental and geographic gradients. Annals of the Missouri Botanical Garden 75, 1-34.

Gigon, A. 1971. Vergleich alpiner Rasen auf Silkaat- und auf Karbonatboden. Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, Zürich 48, 1-163.

Grime, J.P. (1965). The ecological significance of lime-chlorosis. New Phytologist 64, 477-487.

Grubb, P.J. (1977). The maintenance of species richness in plant communities: the importance of the regeneration niche. Biological Review, 52, 107 - 145.

Grubb, P.J., Green, H.E. & Merrifield, R.C.J. (1969). The ecology of chalk heath: its relevance to the calcicole-calcifuge and soil acidification problems. Journal of Ecology, 57, 175-212.

Hamrick, J.L. & Lee, J.M. (1987). THe effect of soil surface topography and litter cover on the germination, survival, and growth of musk thistle (Carduus nutans). American Journal of Botany 74, 451-457.

Harper, J.L. (1977). Population Biology of Plants. Academic Press, London.

Harper, J.L. & Benton, R.A. (1966). The behaviour of seeds in the soil. II. The germination of seeds on the surface of a water supplying substrate. Journal of Ecology 54, 151-166.

Harper, J.L., Williams, J.T. & Sagar, G.R. (1965). The behaviour of seeds in the soil. 1.The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. Journal of Ecology, 53, 273 - 286.

Hegarty, T.W. (1977). Seed activation and seed germination under moisture stress. New Phytologist 78, 349-359.

Hodgson, J. (1989). Are families of flowering plants ecologically specialised? Plants Today July-August, 132 - 138.

Jefferies, R.L. & Willis, A.J. (1964). Studies on the calcicole - calcifuge habit. II. The influence of calcium on the growth and establishment of four species in soil and sand cultures. Journal of Ecology, 52, 691 - 707.

Karamos, A.J. (1981). The development of water deficits in plants. From: Simpson, G.M. (Ed.), Water Stress on Plants. Praeger Publishers.

Keddy, P.A. & Constabel, P. (1986). Germination of ten shoreline plants in relation to seed size, soil particle size and water level: an experimental study. Journal of Ecology 74, 133-141.

Kruger, F.J. & Taylor, H.C. (1979). Plant species diversity in Cape fynbos. Gamma and delta diversity. Vegetatio 41, 85-95.

Lamont, B. B., Hopkins, A.J.M. & & Hnatiuk, R.J. (1984). The flora composition, diversity and origins. From: Pate, J.S. & Beard, J.S. (Eds). Kwongan, Plant Life of the Sand Plain. University of Western Australia Press, Nedlands.

Linder, H.P. (1985). Gene flow, speciation and species diversity patterns in a species-rich area: the Cape flora. From: Vrba, E.S. (Ed.), Species and speciation, 53-57.

Mayer, A.M. & Poljakoff-Mayber, A. (1975). The Germination of Seeds. Pergamon Press.

Mazer, S.J. (1987). Seed mass of Indiana dune genera and families: taxonomic and ecological correlates. Evolutionary Ecology 4, 326-357.

Norton, C.R. 1987. Introduction to the Symposium. HortScience 21, 1104.

Pate, J.S., Rasins, E., Rullo, J. & Kuo, J. (1985). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. Annals of Botany, 57, 747-750.

Reader, R.J. & Buck, J. (1986). Topographic variation in the abundance of Hieracium floribundum: relative importance of differential seed dispersal, seedling establishment, plant survival and reproduction. Journal of Ecology 74, 815-822.

Ronsheim, M.L. (1988). Determining the pattern of resource allocation in plants. Trends in Ecology and Evolution 3, 30-31.

Rorison, I.H. (1967). A seedling bioassay on some soils in the Sheffield area. The Journal of Ecology, 55, 725 - 740.

Rourke, J.P. (1980). The Proteas of Southern Africa. Purnell, Cape Town.

Salisbury, E. (1974). Seed size and mass in relation to the environment. Proceedings of the Royal Society of London 186 83-88.

Shmida, A. & Ellner, S. (1984). Coexistence of plant species with similar niches. Vegetatio 58, 29-55.

Taylor, H.C. (1978). Capensis. In M.J.A.Werger, Biogeography and Ecology in Southern Africa 1, 171-229. The Hague.

Thwaites, R.N. & Cowling, R.M. (1988). Landscape - vegetation relationships on the Agulhas Plain, South Africa. Catena, 15, 333 - 345.

van Staden, J. (1966). Studies on the germination of seed of Proteaceae. South African Journal of Botany 32, 291-298.

van Staden, J. & Brown, N.A.C. (1977). Studies on the germination of South African Proteaceae - a review. Seed Science and Technology 5, 633-643.

van Wilgen, B.M. & Lamb, A.J. (1985). The wildflower picking industry in relation to mountain catchment in the fynbos. Acta Horticulturae 185, 181-187.

Wanntorp, H., Brooks, D.R., Nilsson, T., Nylin, S., Ronquist, F., Stearns, S. & Wedell, N. (1990). Phylogenetic aproaches in ecology. Oikos 57, 119-132.

Wentworth, T.R. (1981). Vegetation on limestone and granite in the mule mountains, Arizona. Ecology 62, 469-482.

Williams, I.J.M. (1972). Contributions of the Bolus Herbarium No. 3. A revision of the genus Leucadendron (Proteaceae).

Section 2

2.0 THE ROLE OF THE REGENERATION NICHE IN DETERMINING ADULT DISTRIBUTION PATTERNS

2.1 ABSTRACT

The control of the distinct, natural distributions of two species-pairs was investigated. _Protea obtusifolia_ and _Leucadendron meridianum_ occur on shallow soils overlying limestone, and _P.susannae_ and _L.coniferum_ on the adjacent, deep, weakly acidic colluvial sands. The hypothesis that regeneration niche requirements at the seed germination, and/or seedling establishment stages determine the adult distribution patterns, was tested. Seed reciprocal transplants were performed in the field. Seedling emergence, establishment and mortality, as well as soil temperature and moisture, were monitored over two summers. Iron, aluminium, calcium and magnesium contents of one year-old shoots were determined. Mid-summer water stress measurements of seedlings were obtained. There was higher seedling emergence of seed of all species on the limestone soils, negating the idea that seed had germination requirements specific to their natural soil types. This higher emergence was suggested to be related to the higher moisture contents of the limestone soils. _Protea obtusifolia_ and _Leucadendron meridianum_ seedlings performed better on their own soil type. By the end of the second summer they had increased seedling mortality on the colluvial sands, and overall increased survival (taking into account seed germination and seedling establishment) on their own soil type. _Protea susannae_ and _Leucadendron coniferum_ seedlings, on the other hand, had slightly increased seedling mortality, and overall less survivorship on their own soil type. Despite this, seedlings of these two species growing on the colluvial sands were larger than their counterparts on the

limestone soils. There were both more, and larger seedlings than those of P.obtusifolia and L.meridianum on the colluvial sands. It is predicted that under crowded seedling conditions, the former would outcompete the latter. These results showed that the adult distributions could partly be explained by seedling requirements. Niche parameters of these seedlings were not defined, though soil nutrients and moisture contents were discussed.

2.2 INTRODUCTION

Dominant, overstorey Proteaceae shrubs in the fire-prone fynbos vegetation of the Agulhas Plain, South Africa are often edaphically restricted (Cowling et al. 1988). Protea obtusifolia Beuk ex Meisn. and Leucadendron meridianum I.Williams grow on the shallow, alkaline soils overlying limestone, whilst P.susannae Phill. and L.coniferum Meisn. grow on adjacent, deep, weakly acidic colluvials sands derived from limestone (Thwaites & Cowling 1988). Individuals of these two species-pairs are rarely found mixed in the field, indicating strong edaphic specificity.

What controls these distributions? In cultivation mature plants grow well on a wide range of soil types. For example, P.obtusifolia, P.susannae and L.coniferum are successfully cultivated on highly acid soils (Vogts 1982). This suggests that the adult distribution may be determined by processes operating in the seed and seedling stages i.e. during regeneration. Wiens (1976) and Grubb (1977) emphasise the importance of edaphic and microclimatic conditions at the germination and seedling establishment stages in determining adult plant population patterns. In laboratory trials Jefferies & Willis (1964) showed that calcicole seedlings were more vigorous at higher calcium concentrations, and Rorison (1967) found that calcicole species failed to germinate and establish on acid soils. Other workers (Gross 1980, Goldberg 1985, de Jong & Klinkhamer 1988, Collins 1990) have shown that events at seedling establishment determine adult distribution of species occurring in a variety of habitats.

The soils on which these species-pairs of proteoid shrubs occur are different with respect to texture, pH and nutrients (Thwaites & Cowling 1988). Preliminary observations indicate that the limestone soils have higher soil moisture contents than the adjacent colluvial sands. We suggest that these differences would have an effect on both seed germination and seedling establishnent. Germination requirements are often specific (Harper, Williams & Sagar 1965, Mayer & Poljakof-Mayber 1975). A study of two fynbos Proteaceae showed that they had different germination temperature requirements, corresponding to the ecological conditions in which they grow (Brits 1986). It is possible that the study species have germination requirements corresponding to the conditions of the soils on which they occur.

Edaphic differences would also affect seedling establishment. The solubility of heavy metals such as iron, aluminium, calcium and magnesium decreases with increasing pH (Jeffrey 1987, Epstein 1972), and it has been suggested that species tolerant of alkaline soils have efficient iron absorption or utilisation mechanisms (Brown & Jolley 1989). Seedlings not adapted to alkaline soils would develop nutritional deficiencies and suffer high mortality. In addition, nitrogen occurs in different forms according to soil pH (ammonium-N in acid , and nitrate-N in alkaline soils), and it has been shown that ammonium-N adversely affects seedling growth of a calcicole grass species (Rorison 1985).

Investigation into the causes of distribution of calcicole and calcifuge species in Britain and Europe has concentrated on grass species and other herbaceous plants (Jefferies & Willis 1964, Rorison 1967, Hutchinson 1968, Grubb, Green & Merrifield 1969, Gigon 1971). Our study differs from this in that it investigates the distibution of dominant, overstorey woody proteoid shrubs.

In this study I tested the hypothesis that the distribution of these proteoid shrubs would be a result of differences in their regeneration requirements. Field trials were used since there is often poor correlation of laboratory germination with field emergence (Grubb 1977, Norton 1987). I monitored soil temperature and moisture, seedling emergence, establishment and mortality of all species on their own and adjacent soil types, over two summers. In order to understand the processes associated with differential mortality patterns, I determined mid-summer water stress levels of the seedlings on both soil types, and investigated for nutritional deficiencies of the seedlings by analysing shoots for iron, aluminium, calcium and magnesium.

2.3 METHODS

2.3.1 Study species and site description

The proteoid (1.5 - 3.0m high) shrubs studied here regenerate after fire from seed stored in woody cones on the plant canopy (serotiny). Seed released after fire germinate *en mass* during the following rainy winter period.

The study was performed on post-fire soils overlying Mio-Pliocene limestone of the Bredasdorp Formation, and the juxtaposed deep, limestone-derived colluvial sands, at Hagelkraal on the Agulhas Plain (34^{o} 41' S, 19^{o} 33' E). The soils overlying limestone are variably shallow (up to 30cm) and often occur in pockets in the eroded limestone bedrock. The limestone soils have higher clay and silt contents, pH, total nitrogen, combustible carbon, and total phosphorus levels, than the colluvial sands. They also have higher sodium, potassium, calcium, magnesium, iron and aluminium concentrations (Table 2.1).

The climate of the area is mediterranean with 69% of the mean annual rainfall (546 mm) occuring between April and September.

2.3.2 Seed planting

Seed was obtained from cones collected during late summer 1987 from plants of all four study species growing in the study area . Cones were oven-dried at 40^{o}C to promote seed release. Seed was planted to a depth of 2mm in reciprocal transplants during early May 1987, five weeks after the mature vegetation had been burnt. In each of ten plots on both the limestone and adjacent colluvial sands, sixteen seed per species were planted in rows such that seed within and between rows was about five cm apart. These spacings were chosen to avoid seedling interactions. Plots were randomly positioned within an area of approximately 20m x 10m on each soil type. Wire mesh enclosures were erected to prevent seed and seedling predation by vertebrates.

Table 2.1. Post-fire topsoil characteristics of limestone and colluvial sands supporting different Proteoid Fynbos communities at the study site on the Agulhas Plain. Analyses were performed by the Soil Science Section of the Winter Rainfall Region, Department of Agriculture and Marketing. Data are means (SE). *n*=10

Soil type	Limestone	Colluvial sands
Dominant proteoid shrubs	*Protea obtusifolia* *Leucadendron meridianum*	*Protea susannae* *Leucadendron coniferum*
Clay (%)	4.8 (0.8)	0
Silt (%)	7.8 (0.6)	1.0 (0)
Fine sand (%)	52.0 (3.2)	57.8 (2.6)
Medium sand (%)	29.9 (3.4)	40.5 (2.6)
Coarse sand (%)	5.5 (0.7)	0.7 (0.2)
pH	8.0 (0.03)	6.3 (0.2)
Total phosphorus (mg/kg)	168.9 (26.7)	12.5 (4.2)
Organic carbon (%)	4.9 (0.4)	1.3 (0.2)
Nitrogen (%)	0.21 (0.03)	0.03 (0.00)
Sodium (mg/Kg)	36.8 (13.8)	4.6 (0.9)
Potassium (mg/Kg)	46.9 (7.8)	15.6 (3.9)
Calcium (mg/Kg)	4588.0 (668.0)	476.0 (48.0)
Magnesium (mg/Kg)	228.1 (61.0)	45.1 (3.7)
Iron (mg/kg)	1136.0 (135.0)	90.0 (16.0)
Aluminium (mg/kg)	1999.0 (121.0)	157.0 (56.0)

2.3.3 Soil temperatures and moisture levels

An MCS 120-02 environmental data logger (MCS, Cape Town, South Africa) was used to monitor soil surface temperature and moisture levels in each soil type during the first winter when germination occurred. Subsequently moisture probes were buried between 10 and 15 cm below the soil surface in order to monitor the moisture levels encountered by the growing seedling roots. The MCS Nylon Soil Moisture sensors were calibrated to give water content measurements by inserting the sensors in soil samples of the two soil types in trays in the laboratory. The soils were saturated and sensor readings were taken as the soil dried to known water contents.

2.3.4 Seedling monitoring

Cumulative seedling emergence was quantified at two to four weekly intervals until November 1987. Thereafter, and until February 1989, seedling mortality and seedling heights were monitored at one to three monthly intervals.

Ten shoots of one-year old seedlings of each species on each soil type were cut off at ground level, air dried at 80°C for three days, and weighed. Iron, calcium, magnesium and aluminium concentrations of all shoots were determined by by atomic absorption on a hydrochloric acid digestate of the shoot ashes.

The water status of all seedlings was quantified in February (late summer) 1989. Pre-dawn and mid-day xylem pressure potentials were determined using a pressure chamber (PMC Instrument Co. Oregon, USA). Mid-day leaf stomatal conductances and transpiration rates were determined using an LI-1600 steady state porometer (LI-COR, Inc. Nebraska, USA).

2.3.5 Statistical analyses

Two-way analysis of variance was performed on seedling emergence, shoot heights and dry weights, leaf conductances and transpiration rates, in order to determine the influence of soil type and species on these properties. Percentages were transformed by the arcsine of the square root. For each species, Students *t*-tests were used to determine the effect of soil type on shoot metal concentrations. Because sample sizes were small, non-parametric two-way analysis of variance (Zar 1984) was performed on summer-stressed seedlings in order to gauge the effect of soil type and species on pre-dawn (n=4) and mid-day (n=8) water potentials. Statgraphics software (Statgraphics 1987, Inc, USA) was used.

2.4 RESULTS

2.4.1 Seedling emergence

Seedlings began to emerge about forty days after planting in May. Most emergence occurred between mid-June and the end of July, with a few additional seedlings appearing until the end of August. There was significantly higher emergence on the limestone soils than on the colluvial sands, with each species having about 20% more seedlings emerging per plot than on the colluvial sand (Table 2.2). The significant species effect is most likely due to the greater emergence of *Protea susannae* seedlings on both soil types. The mean weekly soil surface

Table 2.2. Relationship between soil type and seedling emergence in reciprocal transplants of seeds of *Protea susannae, P.obtusifolia, Leucadendron coniferum* and *L.meridianum*. Seed of all species were planted during May, five weeks after fire, in wire-protected enclosures in two soil types: colluvial sands, and limestone (16 seed per species per enclosure ; 10 enclosures per soil type). After six months, total numbers of emerged seedlings in each enclosure were expressed as a percent of seed planted. Data are mean percentages (SE). *n*=10 enclosures.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Colluvial sands	59.4 (8.3)	48.8 (7.4)	46.3 (10.3)	45.6 (10.6)
Limestone	79.4 (4.3)	73.8 (3.1)	66.9 (1.6)	68.1 (5.4)

Two-way analysis of variance on effects of soil type and species on seedling emergence. Plots were nested within soil type. Percentages were transformed (arcsine of the square root).

Source of variation	dF	F	P
Soil type	1	34.4	<0.001
Species	3	4.1	<0.05
Interaction soil x species	3	0.1	NS

NS=Not significant

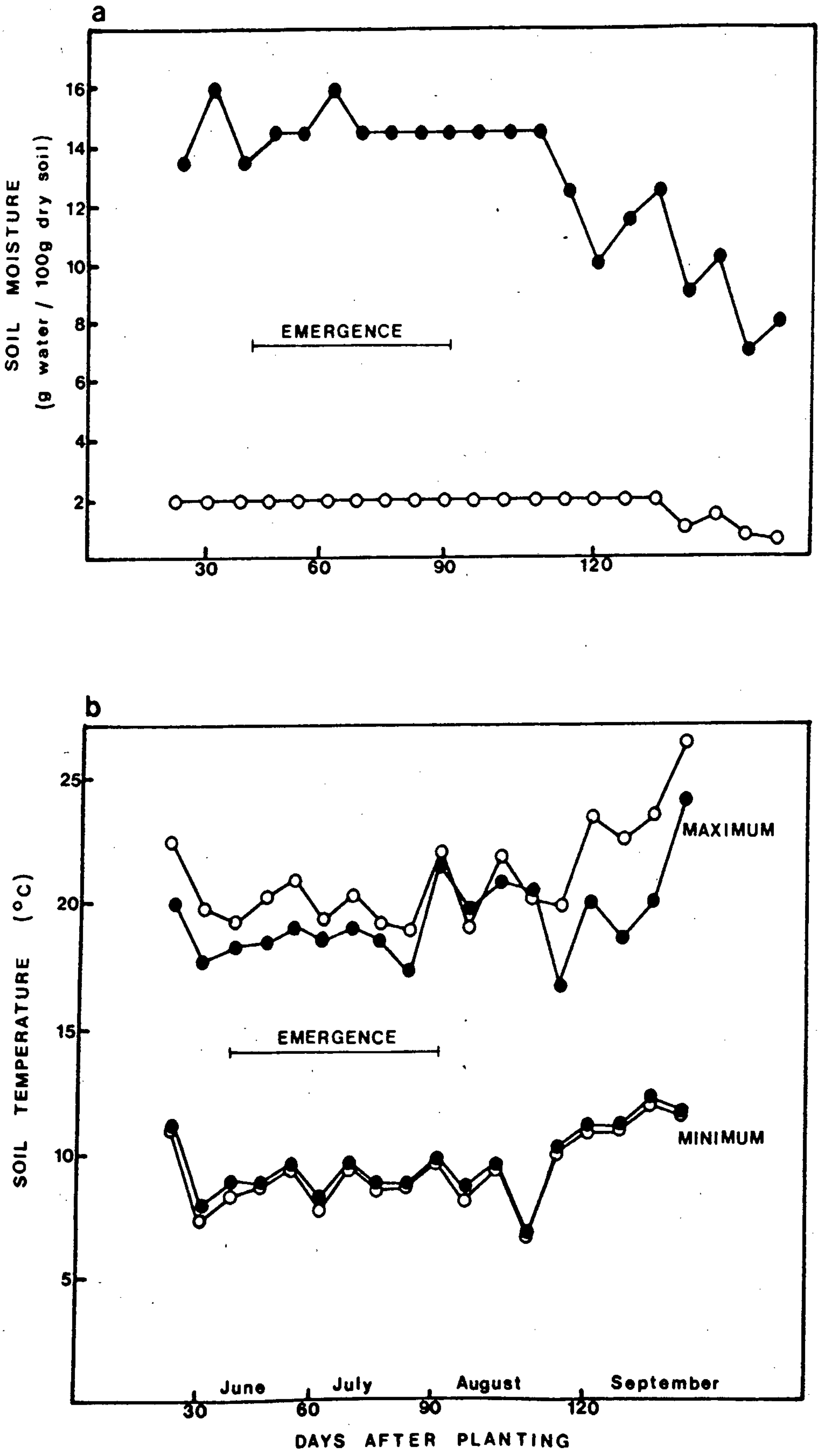


Figure 2.1. a) Moisture contents (g water/100g dry soil) and b) maximum and minimum temperatures of surface soils of colluvial sands (○——○) and limestone (●——●) over the period of seedling emergence (June to September 1987). Measurements were logged on an environmental weather station. Data are weakly means of daily readings.

moisture content throughout the emergence period was 2% (g water/100g dry soil) on the colluvial sands, and between 13.5 and 16% on limestone (Figure 2.1a). During this same time, the mean weekly minimum daily temperatures were similar on both soil types, with a range of 7.3 to 9.9^{o}C (Figure 2.1b). Daily maximum temperatures on the colluvials sands (18.9 to 21.0^{o}C) were one to two degrees higher than limestone soils (17.2 to 19.1^{o}C) during this period.

2.4.2 Seedling growth and mortality

Seedlings of all species were initially taller on limestone than on the colluvial sands, but this trend altered with time (Figure 2.2). Two-way analysis of variance showed that in June 1988 the mean combined seedling heights of all one year-old seedlings growing on the limestone soils were significantly higher ($P<0.001$) than on the colluvial sands. Five months later (November 1988) the mean seedling height of seventeen month-old seedlings on the colluvial sands was higher ($P<0.05$) than that on limestone. The soil x species interaction at this latter time was also significant ($P<0.001$). Seedlings of both Protea susannae and Leucadendron coniferum were taller on their own soil type than on the limestone. By January 1989 this difference had increased. In November 1988 P. obtusifolia and L.meridianum were also taller on their own limestone soils than on the colluvial sands. By January 1989 there were no surviving L.meridianum seedlings on the colluvial sands and the few remaining P.obtusifolia plants on this soil were similar in height to their counterparts on the limestone soils.

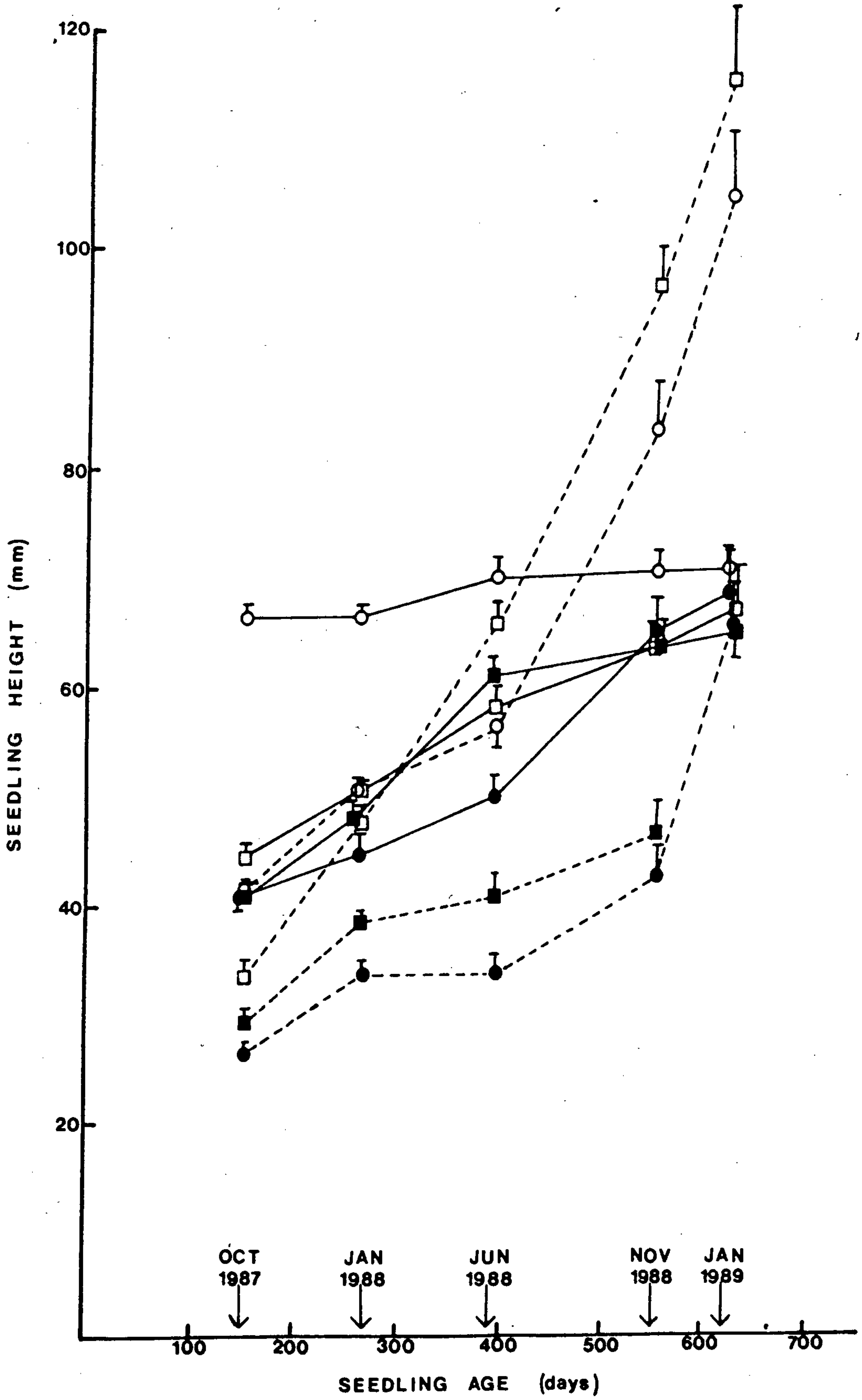


Figure 2.2. Heights (mm) of 4 to 19 month old seedlings of <u>Protea susannae</u> (○), <u>P.obtusifolia</u> (●), <u>Leucadendron coniferum</u> (□) and <u>L.meridianum</u> (■) in seed reciprocal transplants on two soil types: colluvial sands (---------) and limestone (————). Data are means and vertical bars represent standard error of the mean.

Dry shoot mass, a better indicator of plant performance, also showed a significant soil x species interaction in two-way analysis of variance of 17 month-old seedlings, with each species having greater shoot mass on its own soil type (Table 2.3). There was no overall effect of soil type on seedling weight.

For all four species there were more seedling deaths on the colluvial sands than on the limestone by the end of the establishment period (November 1987) (Figure 2.3). Until the end of the following summer (March 1988), approximately equal numbers (ranging from 9 to 13) of seedlings of _Protea susannae_ and _Leucadendron coniferum_ died on both soil types. In contrast, 28 seedlings of _P.obtusifolia_ seedlings and 41 of _L.meridianum_ died on the colluvial sands, giving a mortality 4.7 and 7.0 times higher, respectively, than that on the limestone. Very little rain fell during the following summer (November 1988 - 7mm; December 1988 - 26mm; January 1989 - 5mm; February 1989 - 9mm), and this trend of increased mortality on the colluvial sands continued in the two last mentioned species. This dry summer also resulted in a trebling of seedling deaths of _P.susannae_ (from 11 to 35) and _L.coniferum_ (12 to 38) on their own soil types, whereas total number of deaths on the transplanted limestone soils rose only 1.7 fold in _P.susannae_ and remained unchanged in _L.coniferum_. By the end of the trial, _P.obtusifolia_ and _L.meridianum_ each had four to five times increased mortality on the transplanted colluvial sands. In contrast, _P.susannae_ and _L.coniferum_ showed no increased mortality on the limestone soil, and, in fact, had slightly

Table 2.3. Dry shoot mass (g) of seventeen month-old seedlings of *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum* in reciprocal transplants on two soil types: colluvial sands and limestone. Data are means (SE). *n*=10.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Colluvial sands	0.55 (0.09)	0.23 (0.05)	0.39 (0.06)	0.19 (0.02)
Limestone	0.32 (0.02)	0.35 (0.03)	0.20 (0.02)	0.27 (0.05)

Two-way analysis of variance on effects of soil type and species on dry shoot mass of one year old seedlings.

Source of variation	dF	F	P
Soil type	1	2.87	NS
Species	3	6.50	<0.001
Interaction soil x species	3	7.03	<0.001

NS=Not significant

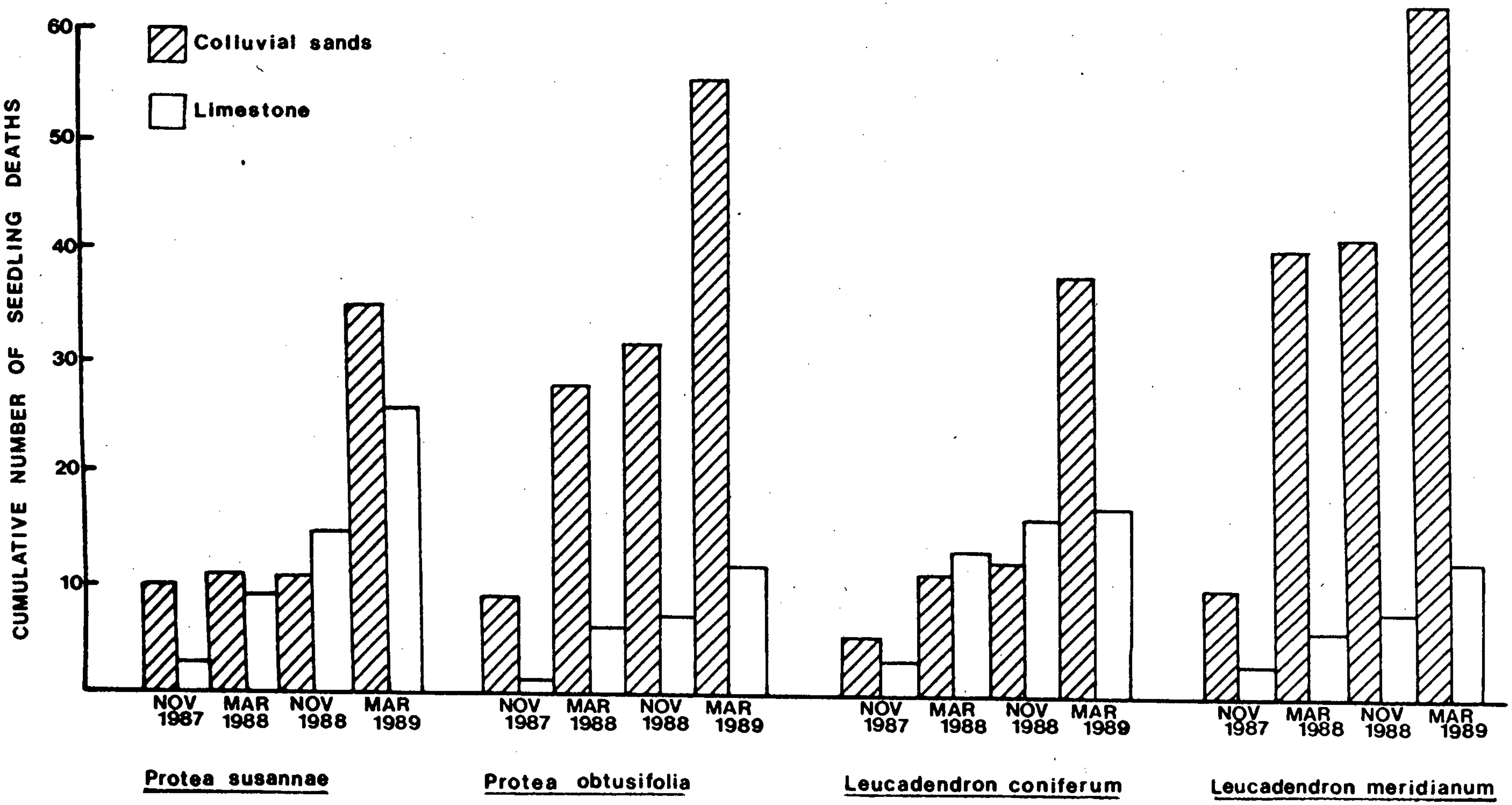


Figure 2.3. Cumulative number of seedling deaths after seedling emergence in winter 1987, of <u>Protea susannae</u>, <u>P.obtusifolia</u>, <u>Leucadendron coniferum</u> and <u>L.meridianum</u> in seed reciprocal transplants on two soil types: colluvial sands and limestone. 160 seed (16 seed in 10 plots) of each species were planted on each soil type.

higher mortality (1.3 and 2.2 times respectively) on their own soil type.

Overall survival (the number of seedlings surviving as a percentage of planted seed) at the end of the experiment in February 1989 showed that Protea obtusifolia had 7.4 times higher survival on its own soil type, and Leucadendron meridianum did not survive at all on the colluvial sands (Table 2.4). On the other hand, on their own soil type, survival of P.susannae was half, and L.coniferum one third that of their survival on limestone. However, survival of P.susannae (30.0%) and L.coniferum (17.3%) was higher than P.obtusifolia (8.5%) and L.meridianum (0%) on the colluvial sands. Survival of all species on the limestone soils was similar, ranging between 53.3 and 64.0%.

Between December 1988 and February 1989 the soil moisture contents of the colluvial sands (15 cm depth) ranged from <1 to 2% (Figure 2.4). Moisture contents of the limestone soils (10 cm depth) ranged from 3 to 12%, at all times higher than the colluvial sands. During the hottest months January and February, the mean (SE) maximum daily air temperatures were 30.2 (0.5) and 29.3 (0.5)^{o}C respectively. The mean maximum (SE) daily soil surface temperature on the colluvial sands in January was 48.6 (0.8), and in February 43.9 (1.5)^{o}C. Comparable readings on the limestone soils were 48.5 (0.7) and 43.5 (1.2)^{o}C.

Shoot analyses showed that there was no difference in iron and aluminium concentrations in seedlings grown on either soil type (Table 2.5). On the limestone soils calcium concentrations

Table 2.4. Survival of *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum* twenty months after planting in reciprocal transplants on two soil types: colluvial sands, and limestone. Data are seedlings surviving in February 1989, expressed as a percentage of seed planted in May 1987.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Colluvial sands	30.0	8.6	17.3	0
Limestone	61.3	64.0	53.3	57.3

were significantly higher in seedlings of all four species than when growing on the colluvial sands. *Protea susannae* and *Leucadendron coniferum* seedlings had significantly higher concentrations of magnesium on the limestone.

There was no significant difference in water potentials of seedlings measured in late summer (February 1989) on the two soil types at either pre-dawn or mid-day (Table 2.6). There were, however, significant differences between species, with *Leucadendron coniferum* seedlings having higher (less stressed) pre-dawn and midday water potentials than the other two species. *P.obtusifolia* seedlings had the lowest water potentials at mid-day. All species showed a drop in water potential at mid-day, on

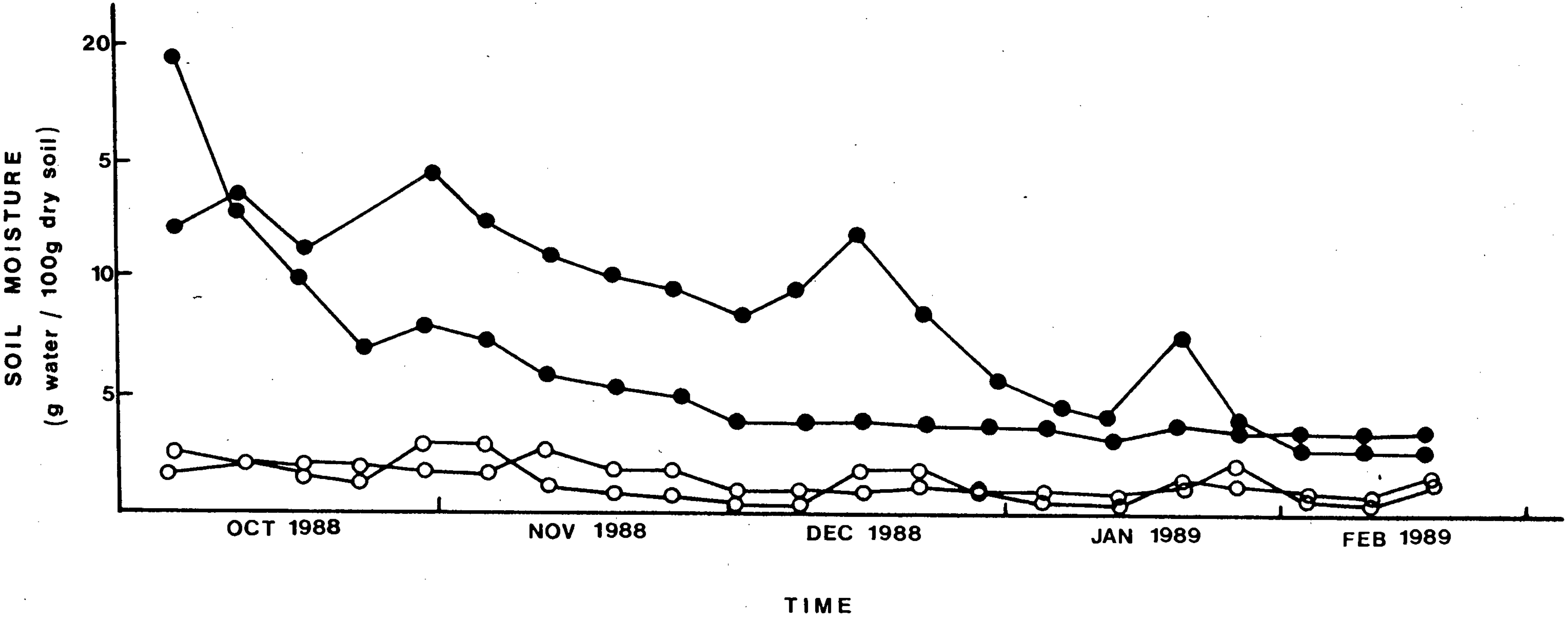


Figure 2.4. Soil moisture contents (g water/100g dry soil) of colluvial sands at 15 cm (○——○), and soils overlying limestone at 10 cm (●——●) depths between October 1988 and February 1989. Two moisture sensors per soil type logged measurements on an environmental weather station positioned in the field.

Table 2.5. Iron, calcium, magnesium amd aluminium concentrations (mg/g dry weight) of one year-old shoots of <u>Protea susannae</u>, <u>P.obtusifolia</u>, <u>Leucadendron coniferum</u> and <u>L.meridianum</u> in reciprocal transplants on two soil types: colluvial sands and limestone. Data are means (SE). *n*=10. The effect of soil type on metal concentrations was determined by Student's *t*-tests.

		<u>Protea susannae</u>	<u>Protea obtusifolia</u>	<u>Leucadendron coniferum</u>	<u>Leucadendron meridianum</u>
Iron	Cs	0.09 (0.02)	0.26 (0.10)	0.17 (0.02)	0.19 (0.03)
	Li	0.10 (0.02)	0.07 (0.02)	0.16 (0.02	0.16 (0.02)
	P	NS	NS	NS	NS
Calcium	Cs	10.09 (0.97)	7.00 (0.54)	11.49 (0.95)	19.42 (0.70)
	Li	15.82 (0.97)	16.34 (1.68)	34.27 (2.20)	25.84 (3.05)
	P	<0.001	<0.001	<0.001	<0.001
Magnesium	Cs	1.31 (0.13)	1.53 (0.13)	3.46 (0.32)	3.83 (0.25)
	Li	1.89 (0.14)	1.50 (0.13)	4.64 (0.20)	4.44 (0.33)
	P	<0.01	NS	<0.01	NS
Aluminium	Cs	0.25 (0.09)	0.51 (0.26)	0.82 (0.36)	0.58 (0.16)
	Li	0.57 (0.20)	0.33 (0.12)	0.60 (0.14)	0.70 (0.20)
	P	NS	NS	NS	NS

NS=Not significant, Cs=Colluvial sands, Li=Limestone both soil types, except for P.susannae on the colluvial sands. There were no significant differences in leaf conductances or transpiration rates for seedlings of all three species on the two soil types (Table 2.7).

2.5 DISCUSSION

2.5.1 Seed germination and seedling emergence

There was no support for the hypothesis that each species would have germination requirements leading to preferential germination and seedling emergence on its own soil type: all four species had higher emergence on the limestone soils. This was probably due to the higher soil moisture contents of this soil type throughout the emergence period - water availability being the major determinant for germination (Mayer & Poljakoff-Mayber 1975). The high moisture contents of these limestone soils would be a consequence of their higher clay, silt and organic matter components. In addition, the limestone rock is porous, retaining up to twenty percent of its weight in water (unpublished data), thus acting as a water reservoir. Keddy & Constabel (1986) also found increased germination in the more moist, finely grained soils along a shoreline gradient, concluding that differential germination requirements were not the cause of vegetation zonation.

Laboratory trials have shown that low temperature (10^{o}C) stimulates germination of these species (Section 3). Since both soil types had similar low minimum temperature ranges of between 7.7 and 9.5^{o}C, the possibility that a specific low temperature

Table 2.6. Relationship between soil type and summer seedling water potentials in reciprocal transplants of <u>Protea susannae</u>, <u>P.obtusifolia</u> and <u>Leucadendron coniferum</u> (no surviving <u>L.meridianum</u> seedlings on the colluvial sands at this time). Seed of all species were planted in winter 1987 on two soil types: colluvial sands, and limestone. Pre-dawn (<u>n</u> =4) and mid-day (<u>n</u>=8) water potentials (MPa) were measured in February 1989. Data are means (SE).

	Colluvial sands		Limestone soils	
	Pre-dawn	Mid-day	Pre-dawn	Mid-day
<u>Protea susannae</u>	-2.83 (1.04)	-2.84 (0.21)	-2.45 (0.43)	-3.79 (0.43)
<u>Protea obtusifolia</u>	-2.78 (0.17)	-4.18 (0.50)	-3.13 (0.27)	-4.01 (0.14)
<u>Leucadendron coniferum</u>	-1.42 (0.17)	-2.38 (0.17)	-1.13 (0.31)	-2.33 (0.26)

Non-parametric two-way analysis of variance on effects of soil type and species on summer stressed seedling water potentials.

		Pre-dawn		Mid-day	
<u>Source of variation</u>	<u>dF</u>	<u>H</u>	<u>P</u>	<u>H</u>	<u>P</u>
Soil type	1	0.03	NS	1.2	NS
Species	2	10.8	<0.001	21.9	<0.001
Interaction soil x species	2	0.6	NS	1.6	NS

NS=Not significant

Table 2.7. Leaf conductances and transpiration rates of 19 month old seedlings of _Protea susannae, P.obtusifolia_ and _Leucadendron coniferum_ in reciprocal transplants on two soil types: colluvial sands and limestone. Measurements were taken at midday during late summer (February 1989). Data are means (SE). _n_=10, except where otherwise indicated.

	Protea susannae	_Protea obtusifolia_	_Leucadendron coniferum_
a) Conductance (mm/s)			
Colluvial sands	7.28 (1.63)	6.61 (1.21)	12.19 (2.71)
Limestone soils	8.08 (1.30)	8.09 (1.43)	9.10 (0.52) (_n_ = 4)
b) Transpiration (mmol/m^{2}s)			
Colluvial sands	0.63 (0.11)	0.58 (0.09)	0.91 (0.16)
Limestone	0.83 (0.12)	0.83 (0.12)	0.95 (0.07) (_n_ = 4)

Two-way analysis of variance on effects of soil type and species on leaf conductances and transpiration rates of seedlings.

	Conductance			Transpiration		
Source of variation	_dF_	_F_	_P_	_dF_	_F_	_P_
Soil type	1	0.02	NS	1	3.04	NS
Species	2	2.58	NS	2	2.11	NS
Interaction soil x species	2	0.71	NS	2	0.26	NS

NS=Not significant

encountered on the limestone soils led to increased germination is eliminated.

2.5.2 Seedling performance and survival

A second hypothesis, that seedling performance and survival of species would be better on its own than on a transplanted soil type, is partially upheld. The 17 month-old seedlings of each species were both taller, and had greater dry shoot weights on their own soils than seedlings of the same species on the transplanted soil type. Laboratory pot-experiments showed that one year-old Proteaceae calcifuge seedlings had lower weights when grown in limestone soils than when grown in acid sands (Newton, Cowling & Lewis, in press). Calcicole Proteaceae seedlings, however, showed no weight differences when grown on either soil type.

The hypothesis is further upheld for the limestone endemics, _Protea obtusifolia_ and _Leucadendron meridianum_, which had increased seedling mortality on the colluvial sands and overall increased survival (taking into account both seed germination and seedling establishment) on their own soil type. _L.meridianum_ suffered total mortality on the transplanted sands, and only 8.6% of _P.obtusifolia_ seeds planted on the colluvial sands survived to the seedling stage after two summers.

Protea susannae and _Leucadendron coniferum_, on the other hand, had slightly increased seedling mortality on their own soil type and, overall, less survivorship by the end of the trial. The performance of these species was not consistent with the prediction of better seedling survivorship on their own soil type. Yet, more of these seedlings survived on the colluvial sands, and they were larger (taller and greater plant biomass) than those of P.obtusifolia and L.meridianum (eliminated after the second summer). They were also larger on the colluvial sands than when they grew on the limestone. In these trials there were no observed competitive effects between seedlings. Nearest neighbour analyses of seedling heights regressed against nearest neighbour heights, were either weakly negative or positive, ranging from -0.02 to 0.82 (unpublished data). With crowded seedlings, as normally occurs after fire, it is expected that P.susannae and L.coniferum would be the sole survivors on the colluvial sands.

All seedlings growing on the limestone soils had enhanced survival - possibly due to the higher moisture levels during the summer growing season. However, since P.susannae and L.coniferum have slightly smaller seedlings than P.obtusifolia and L.meridianum respectively, and since there were indications of chlorosis (many P.susannae seedlings on the limestone soils became yellowed during the last year of the trial (pers. observation), it could well be that the former pair are eventually outcompeted by the latter in later stages of seedling development. Small differences in relative growth rate may translate into a major competitive effect (Givnish 1986). Reciprocal transplants of alpine calcicole and calcifuge grass species by Gigon (1971) on calcareous and acid soils showed less vigorous, chlorotic growth of the calcifuge grass on the former soil than on the latter. However, Gigon's results differed from this study in that the calcicole grass grew equally well on both soils, and it was suggested that the more vigorous calcifuge species would ultimately outcompete the former on acid soils. Even without competitive effects, Protea obtusifolia and Leucadendron meridianum had very low, or no, survival on the colluvial sands. This difference between the two studies could be explained by different moisture regimes on the alpine and Agulhas soils. The alpine calcareous soil are more xeric than the acid soils, contrary to the Agulhas limestone soil having higher moisture contents than the colluvial sands.

2.5.3 Causes of differential seedling performance

Laboratory trials of seedlings grown in acid-washed sand show that the root weights and lengths of Protea obtusifolia and Leucadendron meridianum are less than those of P.susannae and L.coniferum respectively (see Table 5.4). If extrapolated to a field situation this could result in the former two species being unable to obtain sufficient moisture from the drier colluvial sands during the summer months, explaining their high mortality on this soil type. Since the pre-dawn and mid-day water potentials, as well as stomatal conductances and transpiration rates, of the summer stressed seedlings of P.obtusifolia were the same on both soil types, it would appear that water stress was not the major factor causing mortality of this species on the colluvial sands. There were no surviving L.meridianum seedlings to sample. Since no measurements had at any time during the trial been taken on any of the seedlings that had succumbed, and the few survivors would have been the successful ones anyway, this idea cannot be totally discounted.

Soil surface temperatures during summer were similar on the two soil types, discounting differential heat-induced mortality.

Improved seedling growth of the study species on their own soil types could be due to nutritional adaptations. In laboratory experiments calcicole seedlings were most vigorous at high calcium levels, whereas calcifuge species grew well only at low concentrations (Jefferies & Willis 1964). Iron-induced chlorosis at alkaline pH levels is well known, and it is suggested that calcicoles have efficient iron absorption or utilisation mechanisms (Jeffrey 1987). This trial showed no differences in iron concentrations of the seedling shoots of the four study species on the two environments. However, the slightly higher magnesium levels of shoots of Protea susannae and Leucadendron coniferum on the limestone soils, relative to their own soil types, could reflect some metabolic difference. The yellowing of leaves of P.susannae indicate calcium-induced chlorosis. High bicarbonate concentrations in moist calcareous soils have been shown to interfere with root iron metabolism, resulting in decreased chlorophyll synthesis (Hutchison 1968). In general, the range of calcium and magnesium levels of these seedling shoots fell within the range of values reported by Walters (1980) for leaves of mature plants of 116 Proteaceae species, where no correlation between soil type and plant contents of these two cations was found.

Further investigation is needed to provide answers explaining these differential seedling performances on the two soil types. An extreme view suggests that seedlings are unlikely to have adaptations other than phenotypic variability enabling a few seedlings to survive the many factors causing mortality (Fenner 1987). However, the seedlings of the species in this study do appear to have adaptations (as evidenced by differential seedling growth and mortality) to their own soil types.

2.5.4 Conclusions

I have found that the distinct distributions of the two species pairs in this study can partly be explained by regeneration niche requirements. Whilst seed of all species had no germination niche specific to their own soil types, Protea obtusifolia and Leucadendron meridianum seedlings were either unable, or poorly able to survive off their own soil type. On the other hand, seedlings of P.susannae and L.coniferum, whilst being taller and having greater biomass on their own soil type than on the transplanted one, had better survival on the limestone soils than on their own soil type. Since their seedlings on the limestone soils were smaller than those of P.obtusifolia and L.meridianum, it is predicted that they would eventually be outcompeted by the latter.

2.6 REFERENCES

Brits, G.J., (1986). Influence of fluctuating temperatures and H_2O_2 treatment on germination of Leucospermum cordifolium and Serruria florida (Proteaceae) seeds. South African Journal of Botany, 52, 286 - 290.

Brown, J.C. & Jolley, V.D. (1989). Plant metabolic responses to iron - deficiency stress. BioScience, 39, 546 - 551).

Collins, S.L. (1990). Habitat relationships and survivorship of tree seedlings in hemlock-hardwood forest. Canadian Journal of Botany, 68, 790 - 797.

Cowling, R.M., Campbell,B.M., Mustart, P.J., McDonald, D.M., Jarman, M.L. & Moll, E.J. (1988). Vegetation classification in a floristically complex area: The Agulhas Plain. South African Journal of Botany 54, 290 - 300.

de Jong, T.J. & Klinkhamer, P.G.L. (1988). Seedling establishment of the biennials Cirsium vulgare and Cynoglossum officinale in a sand-dune area: the importance of water for differential survival and growth. Journal of Ecology, 76, 393 - 402.

Epstein, E. (1972). Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons.

Fenner, M. (1987). Seedlings. *New Phytologist*, 106 (Suppl.), 35 - 47.

Gigon, A. 1971. Vergleich alpiner Rasen auf Silkaat- und auf Karbonatboden. Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, Zürich 48, 1-163.

Givnish, T.J. 1986. *On the Economy of Plant Form and Function*. Cambridge University Press.

Goldberg, D.E. (1985). Effects of soil pH, competition, and seed predation on the distribution of two tree species.*Ecology*, 66, 503 - 511.

Gross, K.L. (1980). Colonization by *Verbascum thapsus* (Mullein) of an old-field in Michigan: experiments on the effects of vegetation. *Journal of Ecology*, 68, 919 - 927.

Grubb, P.J. (1977). The maintainance of species richness in plant communities: the importance of the regeneration niche. *Biological Review*, 52, 107 - 145.

Grubb, P.J., Green, H.E. & Merrifield, R.C.J. (1969). The ecology of chalk heath: its relevance to the calcicole-calcifuge and soil acidification problems. *Journal of Ecology*, 57, 175-212.

Harper, J.L., Williams, J.T. & Sagar, G.R. (1965). The behaviour of seeds in the soil. 1.The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. Journal of Ecology, 53, 273 - 286.

Hutchinson, T.C. (1968). A physiological study of Teucrium scorodonia ecotypes which differ in their susceptibility to lime-induced chlorosis and iron-deficiency chlorosis. Plant and Soil, 28, 81 - 105.

Jefferies, R.L. & Willis, A.J. (1964). Studies on the calcicole - calcifuge habit. II. The influence of calcium on the growth and establishment of four species in soil and sand cultures. Journal of Ecology, 52, 691 - 707.

Jeffrey, D.W. (1987). Soil -Plant Relationships: an Ecological Approach. Timber Press, USA.

Keddy, P.A. & Constabel,P. (1986). Germination of ten shoreline plants in relation to seed size, soil particle size and water level: an experimental study. Journal of Ecology, 74, 133 - 141.

Mayer, A.M. & Poljakoff-Mayber, A. (1975). The Germination of Seeds. Pergamon Press.

Newton, I.P. Cowling, R.M. & Lewis, O.A.M. (in press). Growth of calcicole and calcifuge Agulhas Plain Proteaceae on contrasting soil types, under glasshouse conditions. South African Journal of Botany.

Norton, C.R. 1987. Introduction to the Symposium. HortScience 21, 1104.

Rorison, I.H. (1967). A seedling bioassay on some soils in the Sheffield area. The Journal of Ecology, 55, 725 - 740.

Rorison, I.H. (1985). Nitrogen source and the tolerance of *Deschampsia flexuosa*, *Holcus lanatus* and *Bromus erectus* to aluminium during seedling growth. Journal of Ecology, 73, 83 - 90.

Thwaites, R.N. & Cowling, R.M. (1988). Landscape - vegetation relationships on the Agulhas Plain, South Africa. Catena, 15, 333 - 345.

Vogts, M. (1982). South Africas Proteaceae. Know them and grow them. Struik.

Walters, C.M. 1980. Die verband tussen substraat en katioonkonsentrasie by die Proteaceae. Unpublished M.Sc. thesis. University of Stellenbosch, South Africa.

Wiens, J.A. 1976. Population responses to patchy environments. *Annual Review of Ecology and Systematics*, 7, 81-120.

Zar, J.H. 1984. *Biostatistical Analysis*. Prentice-Hall Inc.

Section 3

3.0 SEED GERMINATION CHARACTERISTICS

3.1 ABSTRACT

Seeds of Protea susannae, P.obtusifolia, Leucadendron coniferum and L.meridianum were tested for their ability to germinate at different temperatures in the laboratory. Current year seed of all species had more than 90% germination at 10/20°C and 10/10°C. At 15/30°C germination was negligible (1-3%) in all species except Leucadendron coniferum where it was 44%. This could enable L.coniferum to germinate in response to warm season rains after spring burns. In all species canopy-stored seed of increasing age had similarly high germination levels (>89%). Germination rates of older seeds were slower, however, than those of younger seeds, lagging between 2.5 to 6 days behind. This is interpreted as a bet-hedging strategy in response to dry spells after seed release.

3.2 INTRODUCTION

Seedling emergence in fynbos is largely confined to the first winter after fire. Laboratory studies on the germination requirements of Proteaceae seeds have included the effect of inhibitors, promotors, oxygen, hormones and scarification treatments (van Staden 1966, Brown and van Staden 1971, 1973, 1975, van Staden and Brown 1977, Brown and Dix 1985). The above studies were not carried out in an ecological context (e.g. as part of a syndrome of myrmecochory or serotiny) in that the results did not aim to describe processes which explain behaviour in the field.

Recently, laboratory trials have shown a close relationship between the oxygen and temperature requirements of the nut-like seeds of myrmecochorous Proteaceae, and their environments (Brits 1986a & b, Brits 1987). Brits (1986 b) showed that the daily temperature fluctuations required for germination of seeds of the myrmecochorous species Leucospermum cordifolium ($9^{o}C/24^{o}C$) and Serruria florida ($7^{o}C/20^{o}C$) closely resembled the post-fire soil temperatures in their respective habitats. The importance of low temperatures for the successful germination of seeds of serotinous species has been suggested by Bond (1984). Corroborative evidence shows that in the field germination occurs in the cold winter months (Bond 1984, Midgley 1989). Deall and Brown (1981) found that low temperature ($5^{o}C$) incubation significantly improved the germination of Protea magnifica seeds, and Brown and van Staden (1971) found that in three out of five Proteaceae species, higher levels of germination occurred at 15/20°C than at 20/30°C.

Since much ecological importance is attached to the serotinous habit (Lamont et al. 1991), it is important to know the levels of viability of different ages of canopy-stored seeds. Bond (1985) and Cowling et al. (1986) found varying patterns of viability with increasing age of canopy-stored seed for South African and Australian Proteaceae respectively. Le Maitre (1990) found that although germination success of canopy-stored seed did not decline with age, germination rates did.

In this study I performed laboratory tests to investigate germination characteristics of four Agulhas Plain Proteaceae: *Protea susannae* Phill., *P.obtusifolia* Beuk ex Meisn., *Leucadendron coniferum* (L.) Meisn. and *L.meridianum* I.Williams. I tested whether low temperatures alone, or daily fluctuating temperatures, are necessary for germination by incubating seed at 10/20°C, 10/10°C and 15/30°C. I also investigated the viability and germination rates of different ages of canopy-stored seed. The results are interpreted ecologically.

3.3 MATERIALS AND METHODS

3.3.1 Study species

The study species are all non-sprouting, serotinous proteoid shrubs. Their achene-type fruits are commonly referred to as "seed". The species occur as dominants in Proteoid Fynbos of the coastal lowlands of the Agulhas Plain (Cowling et al. 1988). *Protea obtusifolia* and *Leucadendron meridianum* co-occur on the soils overlying limestone, and *P.susannae* and *L.coniferum* co-occur on the adjacent colluvial sands. Seed used in this study was collected from 30 plants per species in 18 year old populations growing in the Heuningrug area, south of Bredasdorp (34^{o} 35/ S, 19^{o} 55/ E).

3.3.2 Methods

Seed of different ages was obtained from cones collected during March 1987. Cones aged 0-1 (current), 2,3,4 years and older for Protea spp., and 0-1, 2,3,4 and 5 years and older for Leucadendron spp., were oven dried at 40^{o}C for 48 hours to promote seed release. The apparently viable (plump and heavy), aborted and predated seed were sorted by hand. In order to assess germination at different temperature regimes, one hundred apparently viable current seeds (10 seeds in ten petri-dishes) were incubated on filter paper with 3 ml water containing 0.075% Benlate (fungicide), simulating optimum moisture conditions. Moisture levels were maintained by the addition of water. The use of fungicide is recommended by Benic & Knox-Davies (1983). Petri-dishes were placed in controlled environment growth cabinets under the following alternating temperature regimes: $10/20^{o}$C, $10/10^{o}$C and $15/30^{o}$C, all under alternating dark/light for 14/10 hours. Germination of different aged seed was determined at $10/20^{o}$C. Germination was taken as the emergence of the radicle. In order to investigate the relationship between germination rate and seed age, the number of germinated seeds was noted at intervals of about four days. After 40 days ungerminated seeds were cut open to establish the presence of an embryo. Germination success was calculated as the total number of seeds germinated as a percentage of apparently viable seeds that had full embryos.

3.3.3 Statistical analyses

One-way analyis of variance was performed on the arcsine of the square root transformations (Zar 1984) of the percent germination data. Two-way analysis of variance was used to determine the effect of increasing age of canopy stored seed of each of the four species, on the time taken to germinate. Statgraphics software (Statistical Graphics Corporation) was used for these analyses. The Kolmogorov-Smirnoff two-sample test (Siegel 1956) was used to determine differences in the cumulative germination distributions over time, of seed of increasing age.

3.4 RESULTS

Current seed of all four species had equally high (>90%) germination success at both the 10/20°C and 10/10°C temperature regimes (Table 3.1). At the higher temperatures of 15/30°C, germination was negligible in all species except for Leucadendron coniferum when 44.4% of the seed germinated.

Within each species, canopy-stored seed of all age categories also had equally high (>90%) germination, with the exception of four year-old seed of Protea obtusifolia which had significantly lower germination (89%) than the younger age categories (Table 3.2). Despite these similarities in final germination percentages, there were differences between age categories in cumulative germination distributions over time, with older seed generally lagging behind younger seed by 2.5 to 6 days. Current seed germination of P.susannae had a significantly different

Table 3.1. Percent germination of the current year's seed of *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum* at different temperature regimes. One hundred seed of each species were incubated on moistened filter paper in petri dishes (10 seed per dish) in controlled environment growth chambers at the following alternating temperatures at a 14 hr dark/10 hr light regime: 10/20°C, 10/10°C and 15/30°C. Data are mean (SE) percent seed germinating per petri dish. Significant differences between species at each temperature regime were determined by one-way analysis of variance. Data were transformed to the arcsine of the square root. Different letters (a,b,c,d) indicate significant differences according to Tukeys multiple range test. *n* = 10 petridishes.

	10/20°C	10/10°C	15/30°C
P.susannae	93.9 (3.1)	95.0 (3.1)^{ab}	1.0 (1.0)^{a}
P.obtusifolia	99.0 (1.0)	98.0 (1.3)^{ab}	3.0 (1.5)^{a}
L.coniferum	96.0 (1.6)	99.0 (1.0)^{b}	44.4 (5.0)^{b}
L.meridianum	92.6 (2.6)	90.6 (2.8)^{a}	2.0 (1.3)^{a}
	NS	*	***

NS = not significant
* = $P<0.05$
*** = $P<0.001$

Table 3.2. Percent germination of canopy-stored seed of increasing age of _Protea_ _susannae,_ _P.obtusifolia,_ _Leucadendron_ _coniferum_ and _L.meridianum_. One hundred seed of each species were incubated on moistened filter paper in petri dishes (10 seed per dish) in controlled environment growth chambers at a 10/20°C:14 hr dark/10hr light, regime. Data are mean (SE) percent seeds germinating per petri dish. For each species significant differences between years were determined by one-way analysis of variance. Data were transformed to the arcsine of the square root. Different letters (a,b) indicate significant differences according to Tukey's multiple range test. _n_ = 10 petridishes.

Time stored on plant (years)	_P.susannae_	_P.obtusifolia_	_L.coniferum_	_L.meridianum_
0 - 1	93.9 (3.1)	99.0 (1.0)^{a}	96.0 (1.6)	92.8 (2.6)
2	97.0 (1.5)	99.0 (1.0)^{a}	98.0 (1.3)	95.0 (2.2)
3	99.0 (1.0)	99.0 (1.0)^{a}	93.9 (2.2)	91.3 (4.1)
4	97.0 (1.5)	89.0 (2.8)^{b}	96.0 (1.6)	94.4 (2.4)
>4	-	-	96.0 (2.2)	95.9 (1.7)
	NS	***	NS	NS

NS = not significant
*** = P<0.001

distribution in time from its four year old seed (Table 3.3). Germination rates of current seed of P.obtusifolia differed significantly from those of older seed categories, and Leucadendron coniferum current seed differed from its three, four and five year old seed. There were also different distributions between two year, and both four and five year old seed, as well as between four and five year old seed in L.meridianum. In all these instances, seed within older age categories took longer to germinate than the younger seed. This is reflected in the time taken for fifty percent of seeds to germinate (Table 3.4). There were highly significant differences between age categories of the combined species, with the three and four year old seed taking longer than the younger current and two year old seed. Both Leucadendron spp. had significantly faster germination times than the Protea spp. The interaction between age classes and species was also highly significant.

3.5 DISCUSSION

Unlike the daily fluctuating temperatures needed for the germination of two myrmecochorous Proteaceae (Brits 1986a), all four species in this study had equally high germination at a constant level of 10^{o}C, as when incubated at temperatures fluctuating between 10 and 20^{o}C. This fits the suggestion that, as a drought-avoiding mechanism, only low temperature is required for serotinous Proteaceae to germinate (Bond 1984). Since seeds of serotinous species are not stored in the soil, there is no selection for the recognition of a specific post-

Table 3.3. Differences in cumulative germination distributions over time of canopy-stored seed of different ages of *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum*. One hundred seed of each species were incubated on moistened filter paper in petri dishes (10 seed per dish) in controlled environment growth chambers at a 10/20°C:14 hr dark/10hr light, regime. *n* = 10 petridishes. The Kolmogorov-Smirnoff test was used to test for differences between distributions. Only significant relationships are given.

Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Yr 1: yr 4 ***	Yr 1: yr 2 ***	Yr 1: yr 3 ***	Yr 2: yr 3 ***
	Yr 1: yr 3 ***	Yr 1: yr 4 ***	Yr 2: yr 5 ***
	Yr 1: yr 4 ***	Yr 1: yr 5 ***	Yr 4: yr 5 ***

*** = P<0.001

Table 3.4. Germination rates of increasing ages of canopy-stored seed of Protea susannae, P.obtusifolia, Leucadendron coniferum and L.meridianum. Data are times (days) (SE) taken for fifty percent of a test sample to germinate. One hundred seed of each species were incubated on moistened filter paper in petri dishes (10 seed per dish) in controlled environment growth chambers at a 10/20°C:14 hr dark/10hr light, regime. n = 10 petri dishes. The effect of seed age and species on germinaton rates was determined by two-way analysis of variance. Different letters (a,b,c) indicate significant differences between increasing ages of canopy-stored seed for combined species, and between species for combined years, according to Tukey's multiple range test.

Time stored on plant (years)	Protea susannae [b]	Protea obtusifolia [c]	Leucadendron coniferum[a]	Leucadendron meridianum [a]
0 - 1[a]	20.9 (0.5)	25.1 (0.2)	16.9 (0.4)	18.1 (0.5)
2 [a]	20.1 (0.4)	29.3 (0.7)	16.9 (0.3)	16.8 (0.5)
3 [b]	20.9 (0.6)	30.3 (0.5)	19.5 (0.5)	19.4 (0.5)
4 [b]	23.5 (0.3)	31.1 (0.7)	18.7 (0.3)	16.3 (0.3)
>4	-	-	19.9 (0.4)	19.2 (0.4)

Two-way analysis of variance on the effects of age (0 - 4 years) of canopy-stored seed, and species, on the time taken for fifty percent of seed to germinate.

Source of variation	dF	F	P
Age of canopy stored seed	3	24.2	<0.001
Species	3	504.3	<0.001
Age of canopy stored seed x species	9	11.8	<0.001

fire signal (fluctuating temperatures) as is the case for myrmocochorous species (Brits 1986a). In a separate study (Section 2, Figure 2.1b), field monitoring of the post-fire environments in which seed of these study species were planted, showed that seedling emergence occurred within a period when soil surface daily minimum temperatures ranged between 7 and 10°C (June to August), suggesting good agreement between laboratory and field germination trials.

Almost half the seeds of Leucadendron coniferum had less strict dormancy breaking requirements and germinated at a higher temperature regime (15/30°C). If it is assumed that it is the minimum (15°C) temperatures that are controlling germination, then it can be predicted that this species will be able to germinate in the field over longer periods than the other species in this study, providing sufficient moisture is available. Minimum soil temperatures are rarely greater than 15°C throughout the whole year, though maximum temperatures are often greater than 30°C from late spring to early autumn (unpublished data). I predict that this species would be better able to survive after spring burns than the others, since some germination and seedling establishment could occur during the relatively wet spring months. Seeds would thus not be exposed to both heat (Cowling et al 1986) and predation (Bond 1984) over the summer months. The existence of pure populations of this species in certain areas of the Agulhas Plain, when co-existence with Protea susannae is more common, could be explained by a history of spring burning. It would be necessary to monitor seedling emergence after a spring burn to confirm this.

It can be problematic to extrapolate the significance of laboratory findings to germination behaviour in the field. However, of interest was that not only were there differences in germination requirements between species, but there were also differences in germination behaviour within species. The latter was evident in the seed age-specific germination rates, with older seeds generally taking between 2.5 to 6 days longer to germinate. This time lag would enable the older seeds in the seed bank to remain in the early passive imbibition stages, prior to the later drought sensitive growth stages (Hillel 1972). These "slower" seeds would be immune to subsequent desiccation. Variable seed germination (seed polymorphisms, or heterocarpy) has been described as a bet-hedging strategy in variable and unpredictable environments (Cohen 1968, Harper 1977). The need for bet-hedging in arid areas with sporadic rain is easily understood, and is well documented for many species (Mayer and Poljakoff-Mayber,1975). There might appear to be little need of a bet-hedging strategy for Proteaceae species growing in areas with reliable winter rainfall. However, fynbos Proteaceae that are killed by fire are vulnerable to germination setbacks since they rely totally on their seed for re-establishment of post-fire populations. Since seed lie on, or near to, the soil surface, any temperature, wind or drainage induced micro-habitat changes in moisture would have important consequences for the imbibing seed. A spread of germination in time, acting as a bet-hedge against this would optimise germination. Zammit and Westoby (1987) found that seeds of the obligate reseeder Banksia ericifolia germinated over twice the time than that taken by the resprouter B.oblongifolia, and suggest that this is a risk-spreading mechanism to reduce subsequent seedling mortality in areas where rainfall is unpredictable. Since it is the older seed of these proteoid species that has the longer germination times, I propose that the strategy of seed retention of these older seed on the canopy in Proteaceae is a strategy to maximise post-fire germination in drier, more variable environments where little or no inter-fire recruitment occurs. Both Bond (1985) and Cowling and Lamont (1985) have observed a higher degree of serotiny in xeric than in mesic areas for fynbos and south-western Australian Proteaceae respectively.

3.6 REFERENCES

BENIC, L.M. & KNOX-DAVIS, P.S. (1983). Anthracnose of Protea compacta, caused by Colletotrichum gloeosporioides. Phytophylactica 15: 109-119.

BOND, W.J. 1984. Fire survival of Cape Proteaceae - influence of fire season and seed predators. Vegetatio 56: 65-74.

BOND, W.J. 1985. Canopy stored seed reserves (serotiny) in Cape Proteaceae. S. Afr.J. Bot. 51: 181-186.

BRITS, G.J. 1986a. Influence of fluctuating temperatures and H_2O_2 treatment on germination of Leucospermum cordifolium and Serruria florida (Proteaceae) seeds. S. Afr. J. Bot. 52: 286-290.

BRITS, G.J. 1986 b. The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes. S. Afr. J. Bot. 52: 291-293.

BRITS, G.J. 1987. Germination depth vs. temperature requirements in naturally dispersed seeds of Leucospermum cordifolium and L.cuneiforme (Proteaceae). S. Afr. J. Bot. 53: 119-124.

BROWN, N.A.C. & DIX, L. 1985. Germination of fruits of Leucadendron tinctum. S. Afr. J. Bot. 51: 448-452.

BROWN, N.A.C. & VAN STADEN, J. 1971. Germination inhibitors in aqueous seed extracts of four South African Proteaceae. S. Afr. J. Bot. 37: 305- 15.

BROWN, N.A.C. & VAN STADEN, J. 1973. The effect of scarification, leaching, light, stratification, oxygen and applied hormones on germination of Protea compacta R.Br. and Leucadendron daphnoides Meisn. S. Afr. J. Bot. 39: 185-195.

BROWN, N.A.C. & VAN STADEN, J. 1975. Inhibitors are not responsible for poor germination of Protea compacta seed. S. Afr. J. Bot. 41: 225-230.

COHEN, D. 1968. A general model of optimal reproduction in a randomly varying environment. J. Ecol. 56. 219-228.

COWLING, R.M. & LAMONT, B.B. 1985. Variation in serotiny of three Banksia species along a climatic gradient. Aust. J. Ecol. 10: 345-350.

COWLING, R.M., LAMONT, B.B. & PIERCE, S.M. 1986. Seed bank dynamics of four co-occurring Banksia species. J. Ecol. 75: 289-302.

COWLING, R.M., CAMPBELL , B.M., MUSTART, P.J., McDONALD, D.M., JARMAN, M.L. & MOLL, E.J. 1988. Vegetation classification in a floristically complex area: The Agulhas Plain. S.

Afr. J. Bot. 54: 290-300.

DEALL, G.B. & BROWN, N.A.C. 1981. Seed germination in Protea magnifica Link. S. Afr. J. Sci. 77: 175-176.

HARPER, J.L. 1977. Population Biology of Plants. Academic Press, London.

HILLEL, D. 1972. Soil moisture and seed germination. In: Water Deficits and Plant Growth. Vol III Plant Responses and Control of Water Balance. Ed. T.T.Kozlowski. Academic Press.

LE MAITRE, D.C. 1990. The influence of seed aging on the plant on seed germination in Protea neriifolia (Proteaceae). S. Afr. J. Bot. 56: 49-53.

LAMONT, B.B., Le MAITRE, D.C., COWLING, R.M. & ENRIGHT, N.J. 1991. Canopy seed storage in woody plants. Bot.Rev. (in press).

MAYER, A.M. & POLJAKOFF-MAYBER, A. 1985. The Germination of Seeds. Pergamon Press.

MIDGLEY, J.J. 1989. The effects of date of planting on field establishment of serotinous cape Proteaceae. Vegetatio. 79: 185-192.

SIEGEL, S. 1956. Non-parametric Statistics for the Behavioural Sciences. Mc Graw-Hill International Book Company.

VAN STADEN, J. 1966. Studies on the germination of seed of Proteaceae. S. Afr. J. Bot. 32: 291-298.

VAN STADEN ,J. & BROWN, N.A.C. 1977. Studies on the germination of South African Proteaceae - a review. Seed Sci. & Technol. 5: 633 - 643.

ZAMMIT, C. & WESTOBY,M. 1987. Seedling recruitment strategies in obligate-seeding and resprouting Banksia shrubs. Ecology. 68:1984 - 1992.

ZAR, J.H. 1984. Biostatistical Analysis. Prentice-Hall Inc.

Section 4

4.0 SEEDS, SOIL AND WATER: WHERE IT ALL BEGINS

SEEDS, SOIL AND WATER: WHERE IT ALL BEGINS

4.1 ABSTRACT

Factors responsible for the seedling emergence patterns of Protea susannae, Leucadendron coniferum, P.obtusifolia and L.meridianum observed in the field, were investigated in laboratory experiments. The field patterns showed lower, more variable (in space) emergence in all four species in colluvial sands than in the adjacent limestone soil type (higher clay, silt, organic carbon and moisture contents than the colluvial sands). In the colluvial sands both Protea spp. showed less variable emergence than the Leucadendron spp. In the laboratory, seed of all four species was planted in each of the two soil types at the field water-capacities of each soil. After imbibition, a short drying period showed that in all four species less imbibed water was lost in the limestone soil than in the colluvial sands soil. This indicates that the former soil acts as a better buffer to atmospheric drying than the latter soil, and would explain the field emergence patterns. The limestone soil has a higher water-binding capacity than the colluvial sands at similar matric potentials.

Air-drying experiments of imbibed seeds showed that both Protea spp. retained more water than the Leucadendron spp. after two and a half days. This correlates with the higher pericarp:embryo ratios of the former than the latter, suggesting that the relatively thicker pericarps of the Protea spp. helps to minimise water loss. In addition, seeds of the Protea spp. are obconical and have hairs, characteristics known to aid

water-retention, compared to the flattened, glabrous Leucadendron seeds. The higher and less variable field emergence of the Protea spp. than the Leucadendron spp. could be explained by seed water-retention properties determined by different seed morphologies.

Wetting/drying cycles imposed on Protea susannae and Leucadendron coniferum in laboratory experiments (on filter paper in petri-dishes) showed that a drying cycle imposed after a short imbibition period (4 days) resulted in equally high (>90%) germination of both current year and old canopy-stored seed of the two species. In contrast, drying after a long (11 day) imbibition period resulted in high germination of both categories of P.susannae seed (>90%), and lower germination of L.coniferum seed (9.7% for current year, and 38% for old canopy-stored seed). These results could also be attributed to their seed morphology-related water-loss characteristics. The increased germination success of older than current year canopy-stored seed of L.coniferum strengthens the interpretation that differences in germination rates of different ages of canopy-stored seed is a bet-hedging strategy ensuring maximal germination.

Adult distribution patterns are discussed in relation to these seed and early seedling emergence characteristics.

4.2 INTRODUCTION

"At the scale of size of a seed the physical environment is exceedingly heterogenous" (Harper 1977). Germination is stressed by many as a key process in determining plant distribution (Harper, Williams & Sagar 1965, Grubb 1977, Bewley & Black 1978, Symonides, Silvertown & Andreason 1986). Since adult plant requirements differ from those of seed, an understanding of seed ecology is often needed to fully understand adult plant density and distibution patterns.

Water is a basic requirement for seed germination (Mayer & Poljakoff-Mayber 1975, Bewley & Black 1978). Since soils with different textures have different water-binding properties we would expect such different soil types to have different effects on seed germination. There is evidence that differences in soil microtopography effect seed germination through modifying seed-water relations (Harper, Williams & Sagar 1965, Keddy & Constabel 1986, Hamrick & Lee 1987, Fowler 1988). The size and shape of seeds is also suggested to influence water uptake and retention (Harper & Benton 1966, Harper, Lovell & Moore 1970, Winn 1985).

Field reciprocal transplants of seed of four Proteaceae species, Protea susannae, P.obtusifolia, Leucudendron coniferum and L.meridianum, have shown different emergence patterns on soils with different soil textures and water contents (Section 2, Table 2.2). There were also differences in emergence between the Protea spp. and the Leucadendron spp.. The seed of these two genera have different morphologies. This suggests that different soil-water characteristics of these two soils, and differences in seed morphology, influence germination.

Seeds are believed to have a critical hydration level at which germination begins, with subsequent desiccation resulting in loss of viability: prior to reaching these hydration levels seeds can withstand drying (Hillel 1972). Laboratory experiments of these four Proteaceae species showed that different ages of canopy-stored seeds had different germination rates (Section 3). In general seed stored on the canopy for longer periods germinated more slowly than current year seed. Seeds germinating more slowly would be buffered for a longer period against this drying-induced mortality. This has been suggested to be a bet-hedging strategy to ensure maximal germination in response to dry spells after seed release (Section 3).

This study relates these observed field emergence patterns to water-binding characteristics of the soil types, and seed morphology of the different species. Laboratory experiments are used to investigate the hypothesis that soil type and seed morphology play a role in buffering the loss of seed-imbibed water during subsequent drying, and by implication also affect germination. Seed planted in the two soil types at their respective field water-capacties are given a drying treatment in order to determine the amount of seed-imbibed water retained. In addition, the drying rates of imbibed seeds are determined to test for differences that could be attributed to the different seed structures of the species.

The ability of different ages of canopy-stored seed to germinate after wetting/ drying cycles is also investigated. The hypothesis that drying following a short imbibition period will subsequently lead to higher germination success after re-wetting, than drying after a long imbibition period, is tested. This will be investigated on current year and old canopy-stored seed of Protea susannae and Leucadendron coniferum to investigate whether older-stored seed will show higher germination success after the latter treatment (desiccation after long imbibition period) than current year seed.

The findings are related to adult plant distribution patterns.

4.3 MATERIALS AND METHODS

4.3.1 Study species and study area

The Proteaceae species studied are two closely related species-pairs with different seed morphologies growing on adjacent, different soil types. They are dominant, overstorey shrubs in the fire-prone fynbos vegetation of the Agulhas Plain, South Africa ($34^{o}40$'S, $19^{o}40$'E). Protea obtusifolia and Leucadendron meridianum grow on the soils overlying limestone, and P.susannae and L.coniferum on the adjacent, colluvial sands (Cowling et al. 1988). The seed (achenes) of the Protea spp. are obconical and pubescent (Rourke 1980), and of the Leucadendron spp. flattened and glabrous with winged perimeters (Williams 1972). All species retain seed on the canopy (serotiny) until released by fire, usually in the dry summer period. Germination occurs during the wet winter season. Seed released between fires do not develop into seedlings: populations are even-aged.

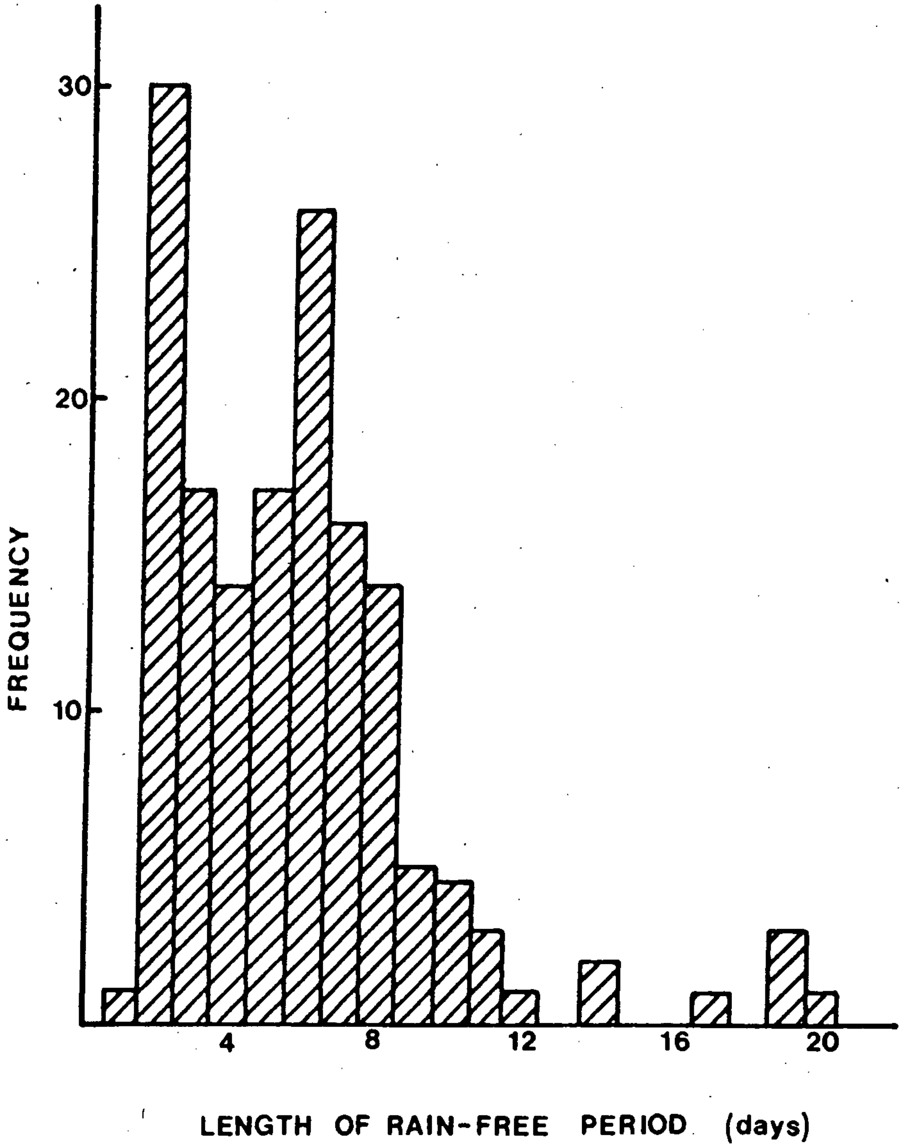


Figure 4.1. Frequency of rain-free periods (1 - 20 days) between May and August. Data were obtained from the Danger Point weather station records from 1982 - 1990.

The climate of the area is mediterranean with 60% of the mean annual rainfall (546 mm) occurring between April and September. During the rainy season when germination occurs (May to August), there are an average of 11 days per month with rain ($\geq$0.1 mm) (data obtained from Danger Point and Cape Agulhas weather stations over the period 1882 - 1984). Figure 4.1 shows the frequency of rain-free periods of 1 to 20 days. The median rain-free period is five days.

Table 4.1 shows the seedling emergence characteristics of the species. Emergence was higher, and less variable in space (lower coefficients of variation) on the limestone soil than on the colluvial sands. In addition, on the colluvial sands emergence was more variable in the Protea spp. than the Leucadendron spp. (Table 4.1b).

4.3.2 Soil texture and soil-water properties

Soil samples were collected from the top 10 cm of soil four months after a February (late summer) fire, and analysed for texture (sand, silt and clay) and organic carbon by the Soil Science Section of the Winter Rainfall Region, Department of Agriculture and Marketing. Soil water contents (g water/ 100g soil) at decreasing matric potentials (-1 to -100 kPa) were determined by the Department of Soil Science, University of Stellenbosch.

4.3.3 Seed weights

Fifty intact seeds, and fifty embryos excised from their pericarps, were weighed. The ratios of pericarp:embryo weights were determined.

Table 4.1. Field emergence characteristics of seed reciprocal transplants of _Protea susannae_, _P.obtusifolia_, _Leucadendron coniferum_ and _L.meridianum_. Seed of all species were planted during May, five weeks after fire, in wire-protected enclosures over an area of 20 x 10 cm in two soil types: colluvial sands, and limestone (16 seed per species per enclosure ; 10 enclosures per soil type). After six months, total numbers of emerged seedlings in each enclosure were expressed as a percent of seed planted. _n_=10 enclosures (from Section 2, Table 2.2).

	Protea susannae	_Protea obtusifolia_	_Leucadendron coniferum_	_Leucadendron meridianum_
a) Mean percent emergence per enclosure (SE)				
Colluvial sands	59.4 (8.3)	48.8 (7.4)	46.3 (10.3)	45.6 (10.6)
Limestone	79.4 (4.3)	73.8 (3.1)	66.9 (1.6)	68.1 (5.4)
b) Coefficient of variation of emergence (%)*				
Colluvial sands	44	46	67	70
Limestone	15	13	7	24

*Calculated from the average and standard deviation of numbers of seed emerging per species per enclosure.

4.3.4 Laboratory trials

These trials were performed under conditions approximating the post-fire soil surface conditions found in the field during winter. All growth chamber incubations in experiments 1a, 1b and 2 were done at 10/20°C under alternating dark/light for 14/10 hours, conditions found to be optimum for the germination of the species (section 3).

The following questions were asked:

1a. Do seeds imbibed in limestone soil retain more water on subsequent drying in this soil, than those planted in colluvial sands soil? Do *Protea* spp. retain more water than the *Leucadendron* spp.?

Colluvial sands and limestone soils were collected from the field, put through a 2mm sieve, and autoclaved. Each soil type was mixed with distilled water to its own winter field capacity of 3.8 and 16.0% respectively. Approximately 4.0g of soil was put into each of the wells of a microtitre plate. Seeds of all four species were weighed, and one seed planted per well. The covered microtitre plates were placed in a growth chamber. Two treatments were performed (n=24 seeds per treatment) as follows:

i. Control treatment with seeds covered for five days.

ii. Drying treatment with the lid removed after three days for 24 hours, and then replaced for one day.

All seeds were then removed from their soil wells, and reweighed after removing adhering soil. In order to account for different seed sizes, the weight of water imbibed was standardised by expressing weight increase as a percent of original seed weight. Soil water contents at the end of the experiment were obtained by determining weight loss after heating at 80°C for 12 hours.

1b. Do seed of species in the different genera have different drying rates?

Ten intact seeds of each species were weighed, imbibed between wet filter paper for two days, and reweighed. Drying rates of the intact seeds were obtained by placing on plastic trays, and drying in a growth chamber. Seeds were weighed at one to two hourly intervals for the first ten hours, and then at daily intervals for one and a half to two days. Weight changes were expressed as a percent of original seed weight.

2. What is the effect of short or long imbibition periods, followed by drying, on germination of current year and older canopy-stored seed of Protea susannae and Leucadendron coniferum?

Current year and old seeds (stored on the canopy for more than four years) of Protea susannae and Leucadendron coniferum were tested for ability to germinate (judged by radical emergence), or, if seeds had germinated prior to final desiccation, by the ability of the radicles to re-expand and re-continue growing in the final wetting stage after imbibition, on filter paper impregnated with 3 ml 0.75% Benlate (fungicide) solution in petri-dishes incubated in a growth chamber. The following treatments were applied to 80 seeds of each species (4 x 20 per petri-dish):

i. Control treatment (normal imbibition in covered petri-dishes).

ii. Drying treatment after short imbibition period: (4 days imbibition in covered petri-dishes followed by 10 days drying in uncovered dishes) - repeated once - followed by normal imbibition.

iii. Drying treatment after long imbibition period: (11 days* imbibition in covered petri-dishes followed by 10 days drying in uncovered dishes) - repeated once - followed by normal imbibition.
(*there was a lag of 16 and 17 days in _Protea_ _susannae_ and _Leucadendron_ _coniferum_ respectively, before germination began in control laboratory trials (unpublished data))

4.3.5 _Statistical_ _analyses_

Three-way analysis of variance was used to determine the significance of soil type, drying treatment and species on water imbibition by seeds, as well as to determine the effect of seed age, drying treatment and species on germination. Germination percentages were transformed to the arcsine of the square root.

4.4 RESULTS

4.4.1 Soil texture and soil-water properties

Table 4.2 shows that the limestone soils have higher clay, silt and organic matter contents than the colluvial sands. The two soil types have markedly different water holding properties, with the limestone soils retaining more water at -5kPa and below, than the colluvial sands (Fig. 4.2).

4.4.2 Seed weights

The *Protea* spp. had greater seed and embryo weights, as well as pericarp:embryo ratios than the *Leucadendron* spp. (Table 4.3).

4.4.3 Laboratory trials

1a. Do seeds imbibed in limestone soil retain more water on subsequent drying in this soil, than those planted in colluvial sands soil? Do Protea spp. retain more water than the Leucadendron spp.?

There were significant differences in the amount of seed-imbibed water between soil type, drying treatment and species (Table 4.4). *Protea susannae* had higher amounts of imbibed water than the other species in all categories. Both *Protea* spp. imbibed more water than the *Leucadendron* spp. in both soil types before drying treatment. Within soil types the effect of drying was significantly different, with less imbibed water in the seeds in the colluvial sand after drying than those in the limestone soils (significant soil type x drying treatment). There was also a significant drying treatment x species effect. Mean percent

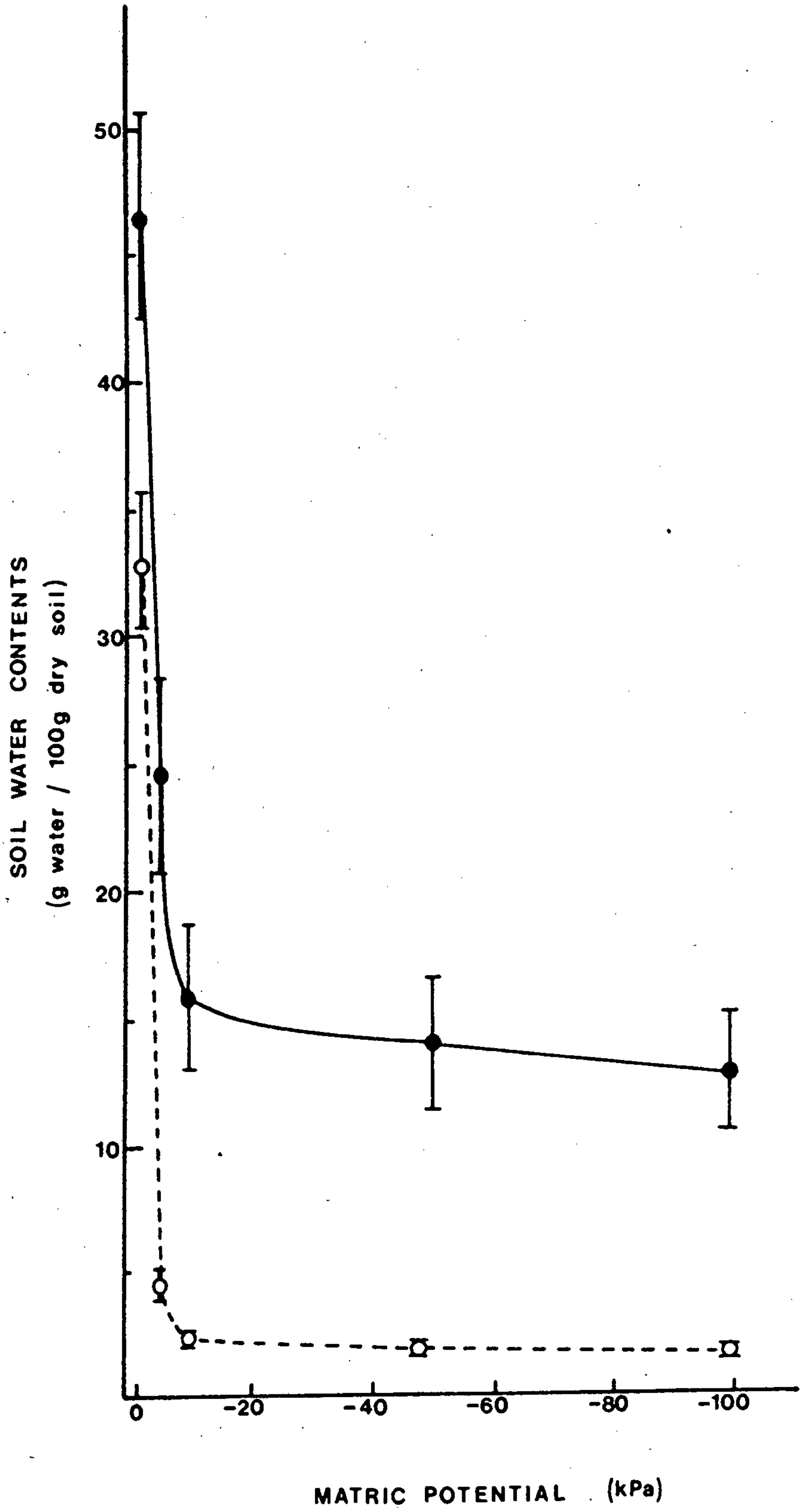


Figure 4.2. Soil water properties of colluvial sands (O---O) and limestone soil (●——●). Soil water contents (g water/100 g soil) at decreasing matric potentials (kPa) were determined by the Department of Soil Science, University of Stellenbosch. Data are means. Vertical bars represent standard errors. n=5.

Table 4.2. Soil texture and organic carbon contents in colluvial sands and limestone soil (0 - 10 cm topsoil). Analyses were performed by the Soil Science Section of the Winter Rainfall Region, Department of Agriculture and Marketing. Data are means (SE). _n_=10.

	Colluvial sands	Limestone
Clay (%)	0	4.8 (0.8)
Silt (%)	1.0 (0)	7.8 (0.6)
Fine sand (%)	57.8 (2.6)	52.0 (3.2)
Medium sand (%)	40.5 (2.6)	29.9 (3.4)
Coarse sand (%)	0.7 (0.2)	5.5 (0.7)
Organic carbon (%)	1.3 (0.2)	4.9 (0.4)

Table 4.3. Seed and embryo weights (mg) (_n_=50), and pericarp:embryo weight ratios (_n_=20) of _Protea susannae_, _P.obtusifolia_, _Leucadendron coniferum_ and _L.meridianum_. Data are means (SE).

	Protea susannae	_Protea obtusifolia_	_Leucadendron coniferum_	_Leucadendron meridianum_
Seed weight	36.4 (0.5)	23.2 (0.6)	12.6 (0.3)	9.0 (0.2)
Embryo weight	16.8 (0.3)	12.0 (0.4)	9.3 (0.3)	6.1 (0.3)
Pericarp: embryo ratio	1.24 (0.04)	1.01 (0.05)	0.35 (0.01)	0.45 (0.02)

Table 4.4. Differences in seed weight (standardised to a percentage of dry seed weight) of Protea susannae, P.obtusifolia, Leucadendron coniferum and L.meridianum incubated in different soils at field water capacities for three days followed by a drying treatment. The experiment was performed in a growth chamber at a 10/20°C :14 hr dark/ 10 hr light, regime. Data are mean percentages (SE). n=24.

		Protea susannae	Protea obtusifolia	Leucadendron coniferum	Leucadendron meridianum
Colluvial sands	1*	50.8(1.0)	42.0(1.0)	31.0(1.3)	36.1(1.6)
	2#	25.2(1.0)	17.3(1.2)	17.1(0.7)	23.0(1.3)
Limestone soils	1*	49.8(1.1)	40.7(1.3)	30.5(0.9)	35.7(1.5)
	2#	34.8(0.7)	23.0(0.8)	23.2(0.6)	23.6(0.9)

Three-way analysis of variance on effects of soil type, drying treatment and species on seed weight increase due to water imbibition.

Source of variation	DF	F	P
Soil type	1	17.4	<0.001
Drying treatment	1	846.8	<0.001
Species	3	126.1	<0.001
2-Way interactions			
Soil type x drying treatment	1	32.0	<0.001
Soil type x species	3	2.4	NS
Drying treatment x species	3	23.4	<0.001

*Control treatment with seeds incubated in soil in micro-titre plates covered with lids for five days.

#Drying treatment with seeds incubated in soil in micro-titre plates covered with lids for three days, uncovered for one day, and covered again for one day.

soil water per 100g dry soil (SE) determinations at the end of the experiment showed that the colluvial sands lost proportionally more water than the limestone soil (colluvial sands changed from the initial 3.8 % to 1.0 (0.1)% ; limestone soil from 16.0% to 6.9 (0.2)%).

1b. Do the seed of the different genera have different drying rates?

The loss of water during the first six hours was rapid in all species (Figure 4.3). Thereafter both Protea spp. retained more water throughout the experiment than the Leucadendron spp..

2. What is the effect of short or long imbibition periods, followed by drying, on germination of current year and older canopy-stored seed of Protea susannae and Leucadendron coniferum?

Whereas Protea susannae had equally high germination in all categories tested, Leucadendron coniferum had lower germination after the drying treatment imposed after the long imbibition period, than in the other treatments (significant species x drying treatment interaction) (Table 4.5). Old canopy-stored seed of the latter had higher germination than current season seed (significant species x seed age interaction).

In the category where the drying treatment was imposed after a long imbibition period, some of the seeds had germinated prior to the second drying. In Protea susannae the number of seeds germinated at this stage ranged from 3 to 9. After desiccation

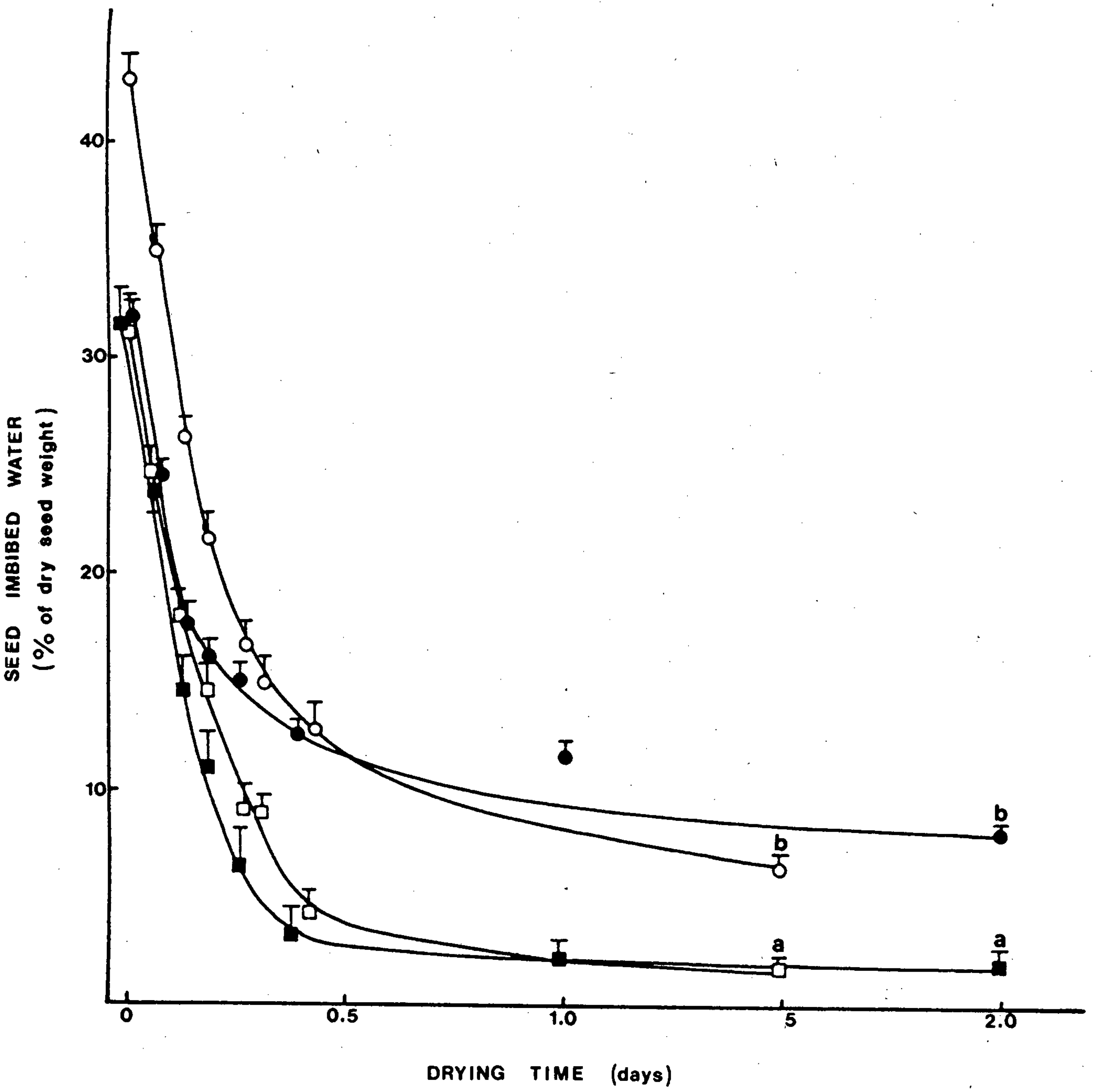


Figure 4.3. Drying rates of imbibed seed of Protea susannae (○), P.obtusifolia (●), Leucadendron coniferum (□) and L.meridianum (■). Seeds were imbibed between wet filter paper for two days, placed on plastic trays and dried at 20/10°C for 10/14 hours. Seed-imbibed water was standardised by expressing it as a percent of original seed weight. Different letters (a, b) indicate significant differences (P<0.001; Tukeys multiple range test between species after one and a half to two days drying).

Table 4.5. Differences in germination of seed of *Protea susannae* and *Leucadendron coniferum* due to canopy-stored seed age and wetting/drying treatments. Seed were incubated on moistened filter paper in petri-dishes at a 10/20°C : 14 hrs dark/ 10 hrs light, regime. Data are means (SE). *n*=4 petri-dishes (20 seed per dish).

	Wetting/ drying treatment		
	Control[+]	Short[$]	Long[Ø]
Protea susannae			
Current seed[*]	98.8 (1.3)	98.7 (1.3)	96.1 (2.5)
Old seed[#]	94.9 (0.1)	94.8 (2.1)	94.8 (2.1)
Leucadendron coniferum			
Current seed[*]	98.7 (1.3)	98.6 (1.4)	9.7 (5.7)
Old seed[#]	98.5 (1.6)	98.5 (1.5)	38.0 (8.3)

Three-way analysis of variance on effects of species, seed age and drying treatment on germination.

Source of variation	DF	F	P
Species	1	41.3	<0.001
Seed age	1	0.1	NS
Drying treatment	2	82.8	<0.001
2-Way interactions			
Species x seed age	1	13.1	<0.001
Species x drying treatment	2	78.8	<0.001
Seed age x drying treatment	2	5.5	<0.01

[*] Seed of current season

[#] Seed stored on the canopy for >4 years.

[+] Inubated in covered petri-dishes.

[$] Short imbibition period followed by drying (4 days imbibition in covered petri-dishes followed by 10 days drying in uncovered dishes) - repeated once.

[Ø] Long imbibition period followed by drying (11 days imbibition in covered petri-dishes followed by 10 days drying in uncovered dishes) - repeated once.

and rehydration these newly emerged radicles re-expanded and continued to grow from 2mm to about 2.0 cm. The low germination obtained by Leucadendron coniferum in this category was largely due to the loss of viability of seeds that had germinated (13 to 19 current seed; 6 to 12 old seed) prior to the second drying. Whereas most of the remaining seeds that had not germinated at this stage, germinated after rehydration, the already emerged radicles of the former did not regain turgidity, and no further regrowth occurred, contributing largely to the low germination values obtained for this species at the end of the experiment.

4.5 DISCUSSION

4.5.1 Soil type, seed morphology and seed-imbibed water

A seed imbibing water on or near the soil surface competes with the drying power of the atmosphere in order to retain sufficient water for germination. This study shows that soil type differs in its effect on buffering seed-imbibed water against drying. Each species imbibed equal amounts of water from the two soils types, yet lost different amounts of water on drying. Each species, except Leucadendron meridianum, retained more water when planted in the limestone soils than in the colluvial sands indicating that the former soil buffers seed-imbibed water loss. The colluvial sands have lower levels of clay, silt and organic carbon compared to the limestone soils, and it is these texture-related differences in pore-size and organic carbon that would lead to the differences in water-holding properties between these two soils (Simpson 1981, Hillel 1982, Jeffrey 1987). The large pore sizes of the colluvial sands lead to rapid drainage. At decreasing, (more negative) matric potentials the water contents of the colluvial sands decrease rapidly to less than 2%. The soil water contents at the end of the seed:soil experiment showed that with the same level of drying (10/20°C for 14/10 hours) the colluvial sands decreased 3.8-fold to 1.0%, whereas the limestone soil diminished two-fold to 6.9%.

Extrapolated to field conditions where rainless periods are frequently longer than the one day used in the experiment (Figure 4.1), this trend found in the laboratory would be enhanced. Similarly, wind, and temperatures greater than 20°C would also strengthen this trend. The finding that field emergence was lower and more variable on the colluvial sands than on the limestone (Section 2) could be explained by the lower water-binding properties of the former soil type than the latter at similar matric potentials. Small changes in microtopography that provide wind and sun shelter or exposure would have major consequences for the retention or loss of water respectively from the surface of this soil, and hence on levels of seed-imbibed water and ultimately on germination. Limestone soils which retain more water than the colluvial sands at similar matric potentials, would be less affected by these factors.

The laboratory finding that Protea spp. retain more water after drying than Leucadendron spp. would explain the less variable emergence patterns of former than the latter on the colluvial sands. These differences in water-retention patterns correlate with the higher pericarp:embryo ratios of the Protea spp. than the Leucadendron spp., suggesting that the thicker pericarp of the former moderates water loss. In particular, P.susannae with the highest ratio has the highest emergence levels on the colluvial sands. In addition, the Protea spp. have other seed characteristics that would help minimise water loss on desiccation. They are round (lower surface area:volume ratio) and have hairs, compared to the flattened (higher surface area:volume ratio), glabrous Leucadendron seed. Flat seeds are particularly sensitive to drying (Harper & Benton 1966).

Under field conditions, where naturally dispersed seed might be only partially covered by soil, the soil effect would be lessened and the seed morphology characteristics might be of relatively greater importance.

Other important factors to be considered in seed-soil water relations are soil hydraulic conductivity, especially important in coarsely-textured soils, as well as the soil particle contact with the seed surface (Bewley & Black 1978). However, laboratory experiments cannot fully simulate or explain field conditions that influence seed-water relations (Hillel (1972).

4.5.2 The effect of wetting/drying cycles on seed germination of current year and older seed of Protea susannae and Leucadendron coniferum.

It appears that the early imbibition stages are resistant to desiccation, and the results indicate that at a critical late stage drying results in loss of viability. Seed of both species had high germination success when subjected to drying cycles after short imbibition periods. This contrasts with the loss of viability of Leucadendron coniferum that had germinated before the final drying cycle imposed with the longer imbibition periods. Germinated Protea susannae seeds in this category resumed growth on re-wetting. Seeds that had not germinated at this stage germinated successfully on re-wetting in both species. This difference between the two species could be attributed to the different seed morphology-related water-loss characteristics discussed above, suggesting that P.susannae seeds are buffered from excess water loss. Alternatively, since L.coniferum germinates quicker than P.susannae (see Section 3, Table 3.4) seedling emergence would have proceeded to a slightly more advanced stage in the former, with the possibilty of increased susceptibility to desiccation. These findings that failure to germinate is associated with failure of early post-emergence rather than with pre-germination is highlighted by others (Hegarty 1977, Matthews & Powell 1986). The increased germination success of the older canopy-stored seed of L.coniferum after the long imbibition/ drying treatment, re-enforces the interpretation that differences in germination rates of different ages of canopy-stored seed could be a bet-hedging strategy to ensure maximum recruitment (Section 3).

4.5.3 Seed ecology and adult distribution

There are many factors contributing to adult plant distribution patterns. I have shown elsewhere that seedling establishment, and not germination requirements, is important in determining the distinctive distributions of these species-pairs on their respective soil types (Section 2). However, rain-free periods co-inciding with critical stages of early seedling emergence would cause large-scale recruitment failure of Leucadendron coniferum, and would be a possible cause of variability of population size. Although Protea susannae was shown to be resistant to emergence failure at this stage, it is possible that the experimental timing of the drying cycle was fortuitous, and if imposed at a slightly later stage of emergence this species would also be adversely effected. The bet-hedging strategy offered by different ages of canopy-stored seed would buffer this susceptibility to emergence failure. Fenner (1987) stresses that the vulnerability of this early post-germination stage is underestimated in demographic studies.

Limits to species distributions can only be explained with full knowledge of germination, establishment and reproductive requirements (Clarke & Durley 1981). The distribution of Leucadendron spp. with different seed morphologies has been suggested to be linked to the amount of annual rainfall (Williams 1972). Increasing concentrations of species with flat, thin-coated fruits, such as those of the study species (section Alatosperma), occur in areas with higher rainfall than areas where greater numbers of species having biconvex, hard-coated, nut-like achenes (section Leucadendron) occur (data in Williams 1972). This fits the suggestion that round seed and a thick pericarp protect seed from water loss. Protea spp. sharing these latter seed characteristics are more widespread in dry fynbos areas than flat, thin-coated *Leucadendron* spp..

4.6 REFERENCES

Bewley, J.D. & Black, M. (1978). Physiology and Biochemistry of Seeds in Relation to Germination. I. Development, Germination, and Growth. Springer-Verlag.

Cowling, R.M., Campbell , B.M., Mustart, P.J., McDonald, D.M., Jarman, M.L. & Moll, E.J. (1988). Vegetation classification in a floristically complex area: The Agulhas Plain. South African Journal of Botany 54, 290 - 300.

Fenner, M. (1987). Seedlings. New Phytologist 106 (Suppl.), 35-47.

Fowler, N.L. (1988). What is a safe site? : neighbour, litter. germination date, and patch effests. Ecology 69, 947 - 961.

Grubb, P.J. (1977). The maintainance of species richness in plant communities: the importance of the regeneration niche. Biological Review 52, 107 - 145.

Hamrick, J.L. & Lee, J.M. (1987). Effect of soil surface topography and litter cover on the germination, survival, and growth of musk thistle (Carduus nutans). American Journal of Botany 74, 451-457.

Harper ,J.L. (1977). Population Biology of Plants. Academic Press, London.

Harper, J.L. & Benton, R.A. (1966). The behaviour of seeds in soil. II. The germination of seeds on the surface of a water supplying surface. Journal of Ecology 54, 151-166.

Harper, J.L., Lovell, P.H. & Moore, K.G. (1970). The shapes and sizes of seeds. Annual Review of Ecology and Systematics 1, 327-356.

Harper, J.L., Williams, J.T. and Sagar, G.R. (1965). The behaviour of seeds in the soil. 1.The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. Journal of Ecology 53, 273 - 286.

Hegarty, T.W. (1977). Seed activation and seed germination under moisture stress. New Phytologist 78, 349-359.

Hillel, D. (1972). Soil moisture and seed germination. In: Water Deficits and Plant Growth. Vol III Plant Responses and Control of Water Balance. Ed. T.T.Kozlowski. Academic Press.

Hillel, D. (1982). Introduction to Soil Physics. Academic Press, Inc. New York.

Jeffrey, D.W. (1987). Soil -Plant Relationships: an Ecological Approach. Timber Press, USA.

Keddy, P.A. & Constabel, P. (1986). Germination of ten shoreline plants in relation to seed size, soil particle size and water level: an experimental study. Journal of Ecology 74, 133-141.

Matthews, S. & Powell, A.A. (1986). Environmental and physiological constraints on field performance of seeds. HortScience 21, 1125-1128.

Mayer, A.M. and Poljakoff-Mayber, A. (1975). The Germination of Seeds. Pergamon Press.

Rourke, J.P. (1980). The Proteas of Southern Africa. Purnell, Cape Town.

Simpson, G.M. (1981). Water Stress on Plants. Praeger Publishers.

Symonides, E., Silvertown ,J. & Andreason, V. (1986). Population cycles caused by overcompensating density-dependence in an annual plant. Oecologia 71, 156-158.

Thwaites, R.N. & Cowling, R.M. (1988). Landscape - vegetation relationships on the Agulhas Plain, South Africa. Catena 15, 333 - 345.

Williams, I.J.M. (1972). A revision of the genus Leucadendron (Proteaceae). Contributions of the Bolus Herbarium No. 3.

Winn, A.A. (1985). Effects of seed size and microsite on seedling emergence of _Prunella vulgaris_ in four habitats. _Journal of Ecology_ 73, 831-840.

5.0 SEED SIZE IN RELATION TO SOIL MOISTURE AND NUTRIENTS: ADAPTATION OR PHYLOGENY ?

- 5.1 Abstract
- 5.2 Introduction
- 5.3 Materials and methods
  - 5.3.1 Study species and soil environments
  - 5.3.2 Seed collection and mass determinations
  - 5.3.3 Phosphorus and nitrogen determinations
  - 5.3.4 Root, shoot measurements, and relative growth rate determinations
  - 5.3.5 Cone, stem and leaf size determinations
  - 5.3.6 Statistical analyses
- 5.4 Results
  - 5.4.1 Seed mass and nutrients
  - 5.4.2 Seedling root and shoot measurements, root:shoot ratios, and relative growth rates
  - 5.4.3 Cone, stem and leaf size
- 5.5 Discussion
- 5.6 References

5.1 ABSTRACT

I studied seed size, seed nutrient status and seedling growth of two closely related fynbos Proteaceae species-pairs growing on juxtaposed soils of different nutrient and moisture status. Seeds had a greater mass and higher phosphorus and nitrogen contents for species occurring on limestone (higher nutrient and moisture contents) than those on the colluvial sands (lower nutrients and moisture). This trend was found within, but not across genera, stressing the importance of phylogeny in interpreting adaptations. It would be difficult to test separately for the effects of either nutrients or moisture, since the same advantage of enhanced seedling size, and hence survival in a stressed environment, applies to both factors. I suggest that soil nutrients and moisture are a selective force determining seed size, and discuss the allometric consequences. The increased root:shoot ratios (using lengths) of the Leucadendron spp. relative to the Protea spp. are interpreted as an attempt to overcome a phylogenetic constraint that results in smaller seed size in the former genus.

5.2 INTRODUCTION

Seed size of flowering plants varies over ten orders of magnitude (Harper, 1977). To some extent seed size variation between species can be attributed to allometry, in that big plants produce big seeds (Thompson & Rabinowitz, 1989). However, there are correlations between seed size (mass) and environmental variation, and it is suggested that differences in seed mass and number are adaptive, resulting from compromises between selection for the successful seedling establishment conferred by larger seeds, and the increased dispersability of smaller seeds (Baker, 1972; Harper, 1977). Seed mass is positively correlated with seedling size (Fenner, 1983; Wulff, 1986; Stock, Pate & Delfs, 1990). Large seedlings perform better than smaller seedlings under density stress (Harper, Lovell & Moore, 1970; Salisbury, 1974; Foster, 1986; Thompson, 1987), and conditions of drought stress (Baker, 1972; Salisbury,1974; Wulff, 1986; Werner & Platt, 1976). This implies selection for large seed size in later successional vegetation, and in dry environments.

It has been proposed that soil nutrients play a role in determining seed size in Proteaceae (Kuo, Hocking & Pate, 1982; Lamont, Collins & Cowling, 1985), with selection for large seeds containing sufficient nutrient reserves for adequate seedling growth in nutrient-poor soils. Esler _et al_., (1989) and Stock _et al_., (1989) have extended this to suggest that the cost of producing large seeds is offset by producing lower numbers of seed. A similar argument would hold for low soil moisture contents acting as a selective force - increased nutrient reserves of large seeds would result in large root systems enabling seedlings to obtain sufficient water for survival.

South African Fynbos Proteaceae grow on a wide spectrum of soil types, and their natural distributions are often edaphically restricted (Cowling, 1990). This allows investigation of the role of soil type in determining regeneration characteristics of seed and seedling size. This study investigates the hypothesis that soil nutrients and moisture contents are selective forces on seed size in four Proteaceae occurring on soil types with different nutrient and moisture characteristics. Since phylogenetic constaints can be a factor determining seed size, I have used two closely related fynbos Proteaceae species-pairs on juxtaposed soils. Seed size (mass), phosphorus and nitrogen contents, as well as laboratory-grown seedling root:shoot ratios and relative growth rates, were determined and interpreted in the light of this hypothesis. The allometric implications of seed size are discussed.

5.3 MATERIALS AND METHODS

5.3.1 Study species and soil environments

The study species are dominant shrubs in fire-prone Proteoid Fynbos communities (Cowling et al., 1988) on the Agulhas Plain, South Africa (34°40'S, 19°40'E). Seed (achenes) are retained on the canopy until released by fire, usually in the dry summer period. Germination occurs during the following wet winter season. Protea obtusifolia Beuk ex meisn. and Leucadendron meridianum I.Williams occur on the shallow soils overlying Mio-

Pliocene limestone of the Bredasdorp Formation, and P.susannae Phill. and L.coniferum Meisn. on the adjacent, limestone-derived colluvial sands (Thwaites & Cowling, 1988). The limestone topoils are richer in nutrients, organic material, clay and silt components (Table 5.1), and have higher moisture contents throughout the year, than the colluvial sands (Figure 5.1). Soil water contents at deeper levels (10 to 15 cm) during the warm season, October to February, are also higher in the limestone soils (range: 3 - 15%) than the colluvial sands (range: 0.5 - 2.5%). The two Protea spp. (Rourke, 1980), and Leucadendron spp. (Williams, 1972) are both closely related in that they occur in the same section of their respective genera. Phylogenetic analyses are lacking, but indications are that they are sister taxa.

5.3.2 Seed collection and mass determinations

Current year cones of all four species were collected during March 1987. These were oven-dried at 40°C for 48 hours to release the seeds. Fifty intact seeds, and fifty excised embryos of each species were weighed.

5.3.3 Phosphorus and nitrogen determinations

Total phosphorus was determined by the digestion method of Jackson (1958) and colour determination of Murphy & Riley (1962). Total nitrogen was determined by the Kjeldahl method (Smith, 1980).

Table 5.1. Characteristics of limestone and colluvial sands (0 - 10 cm topsoil) supporting Proteoid Fynbos communities on different soil types on the Agulhas Plain. Soils were collected after a fire in May 1987. Texture and nutrient analyses were performed by the Soil Science Section of the Winter Rainfall Region, Department of Agriculture and Marketing. For soil nutrients and texture, data are means (SE). _n_=10.

Soil type	Limestone	Colluvial sands
Dominant proteoid shrubs	_Protea obtusifolia_ _Leucadendron meridianum_	_Protea susannae_ _Leucadendron coniferum_
Clay (%)	4.8 (0.8)	0
Silt (%)	7.8 (0.6)	1.0 (0)
Fine sand (%)	52.0 (3.2)	57.8 (2.6)
Medium sand (%)	29.9 (3.4)	40.5 (2.6)
Coarse sand (%)	5.5 (0.7)	0.7 (0.2)
Total phosphorus (mg/kg)	168.9 (26.7)	12.5 (4.2)
Organic carbon (%)	4.9 (0.4)	1.3 (0.2)
Nitrogen (%)	0.21 (0.03)	0.03 (0.00)

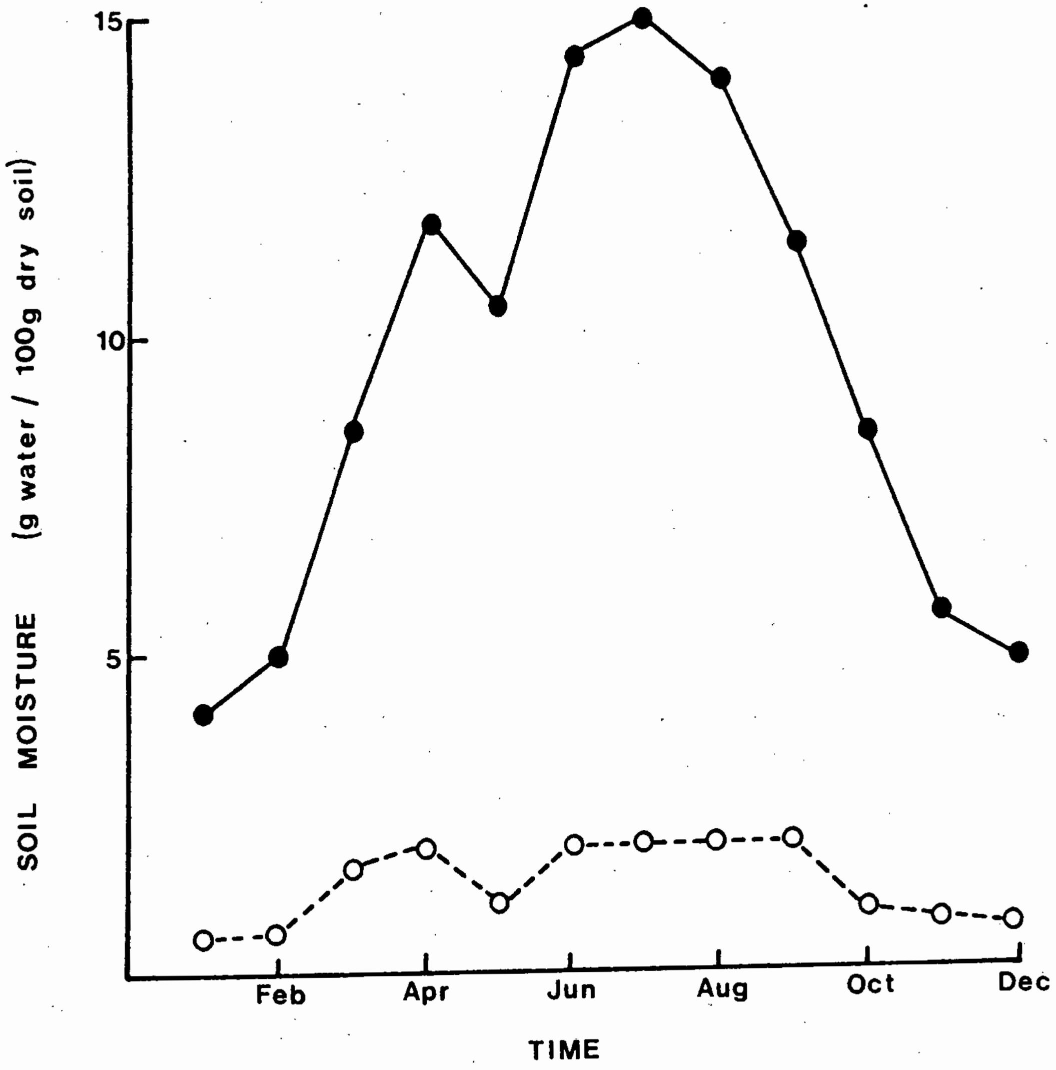


Figure 5.1. Seasonal water contents (g water/100g dry soil) of topsoil (1 - 2 cm depth) of limestone (●) and colluvial sands (○). Measurements were obtained using MCS Nylon Soil Moisture sensors positioned in the field, logging data on an MCS 120-2 environmental weather station. Field capacity of soils overlying limestone was 14.5% and colluvial sands 2.0%.

5.3.4 Root and shoot measurements, and relative growth rates determinations.

Seed were germinated in petri-dishes at 10°C/20°C, and used for transplantation into rooting boxes when the cotyledons had emerged. Rooting boxes (340 cm in depth, and tapering from 120 x 180 cm at the top, to 120 x 80 cm at the bottom), positioned in a greenhouse, were filled with fine, acid-washed sand, saturated with distilled water and allowed to drain overnight. Seedlings (six per species) were randomly allocated one to a box, and planted during August 1988. Equal amounts of water were added to all boxes at regular intervals for the duration of the experiment. Sixteen weeks later (late November) seedlings were harvested, and roots were washed free of sand. Root and shoot lengths were measured. Seedlings were subsequently dried at 50°C for 5 days, and roots and shoots were weighed.

Relative growth rates were calculated as g seedling dry weight/ g embryo/ week according to Hunt (1978) as follows:-
$(\log_e \text{seedling mass} - \log_e \text{embryo mass}) / \text{number of weeks}$.

5.3.5 Cone, stem and leaf size determinations.

Thirty empty cones used for seed collection, and thirty current year, mature leaves collected from several plants all species were dried at 60° for three days and weighed. Stem diameters at the point of attachment to the cone were measured.

5.3.6 Statistical analyses

Two-way analysis of variance was used to determine the significance of seed and embryo mass differences between genera, and between soil types. Kruskal-Wallis analysis was used to test for significant differences between species for seed nutrients, seedling root and shoot measurements, and root:shoot ratios. Wilcoxon's paired-sample test was used to determine the significance of differences between root and shoot relative growth rates.

5.4 RESULTS

5.4.1 Seed mass and nutrients

Seed mass (both whole seeds and embryos) differed significantly with values of the Leucadendron spp. being lower than the Protea spp., and species growing on limestone being lower than those on the colluvial sands (Table 5.2). Whereas there were no significant differences between species for phosphorus and nitrogen concentrations, the total seed contents were different, with the larger seeds having significantly higher nutrient contents (Table 5.3).

5.4.2 Seedling root and shoot measurements, root:shoot ratios, and relative growth rates.

There was a positive relationship between seed and seedling mass (Figure 5.2). Root dimensions of the four species were significantly different, with Leucadendron spp. having longer roots of lower mass than the Protea spp. (Table 5.4). Whilst there was no difference between shoot lengths, shoot mass was significantly different. Using mass, the root:shoot ratios were significantly higher in the Protea spp. than the Leucadendron spp. The reverse was found using length measurements, with

Table 5.2. Differences in a) seed and b) embryo weight between genera (combined *Protea* spp. and combined *Leucadendron* spp.); soil types (*P.obtusifolia* and *L.meridianum* combined, *P.susannae* and *L.coniferum* combined); and interactions between genus and soil type, by two-way analysis of variance. Data are means (SE). *n* = 50. Li = Limestone soil. Cs = Colluvial sands.

Genus		Soil type		Genus x soil type	
a. Seed weight (mg)					
Protea	29.8 (0.8)	Li	16.1 (0.8)	*P.obtusifolia*	23.2 (0.6)
Leucadendron	10.8 (0.3)	Cs	24.5 (1.2)	*P.susannae*	36.4 (0.5)
				L.meridianum	9.0 (0.2)
				L.coniferum	12.6 (0.3)
F	1000.0 ***		408.6 ***		131.3 ***
b. Embryo weight (mg)					
Protea	14.4 (0.3)	Li	9.0 (0.4)	*P.obtusifolia*	12.0 (0.4)
Leucadendron	7.7 (0.2)	Cs	13.0 (0.4)	*P.susannae*	16.8 (0.3)
				L.meridianum	6.1 (0.2)
				L.coniferum	9.3 (0.3)
F	558.1 ***		200.2 ***		8.3 **

**P<0.01, ***P<0.001

Table 5.3. Seed phosphorus and nitrogen contents and concentrations of *Protea obtusifolia*, *Leucadendron meridianum* (limestone) and *P.susannae*, *L.coniferum* (colluvial sands). Data are means. Significance of differences between species was determined by Kruskal-Wallis analysis. *n*=10 (*Protea* spp.) and *n*=5 (*Leucadendron* spp.). dm=Dry mass

	Protea obtusifolia	*Protea susannae*	*Leucadendron meridianum*	*Leucadendron coniferum*	
P content (mg P per seed)	0.12	0.17	0.07	0.07	***
P concentration (mg P g^{-1} dm)	10.7	10.9	12.2	9.0	NS
N content (mg N per seed)	1.29	2.05	0.60	0.93	***
N concentration (g N per g^{-1} dm)	0.12	0.12	0.10	0.10	NS

NS, Not significant. ***$P<0.001$

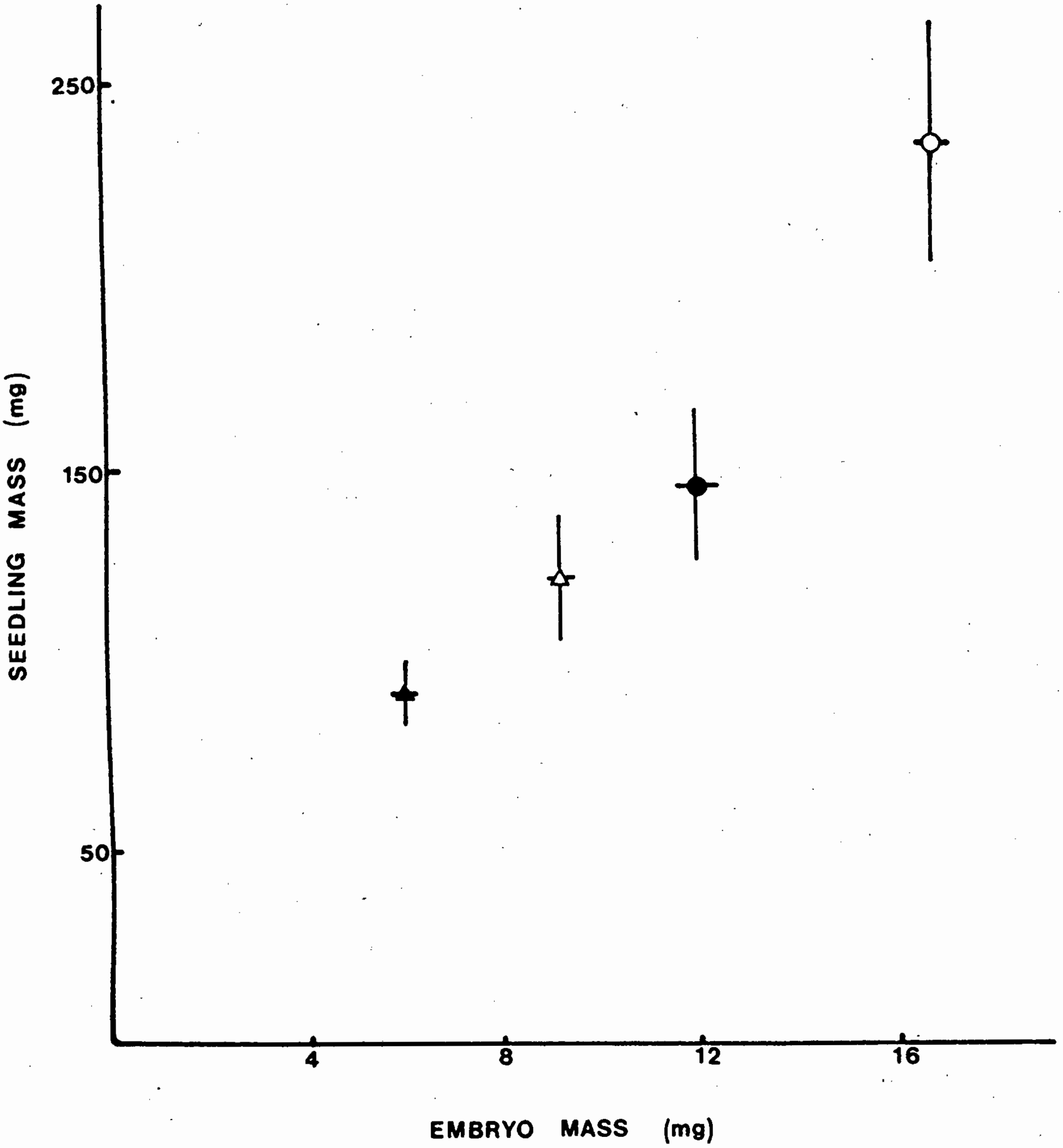


Figure 5.2. Relationship between seed and seedling mass (mg) of Protea obtusifolia (●), Leucadendron meridianum (▲) (limestone) and P.susannae (O), L.coniferum (Δ) (colluvial sands) 16 weeks after growing in root boxes under greenhouse conditions. Horizontal and vertical bars represent standard errors. n=6 (P.susannae and L.coniferum) and n=5 (P.obtusifolia and L.meridianum).

Table 5.4. Seedling root and shoot measurements (length and mass), and ratios of Protea obtusifolia, Leucadendron meridianum (limestone), and P.susannae, L.coniferum (colluvial sands). Seedlings were grown in root-boxes under greenhouse conditions for 16 weeks after germination. Data are means. Significance of differences between species was determined by Kruskal-Wallis analysis. *n*=6 (P.susannae and L.coniferum) and *n*=5 (P.obtusifolia and L.meridianum)

	Protea obtusifolia	Protea susannae	Leucadendron meridianum	Leucadendron coniferum	
Root length (mm)	101.7	145.3	156.7	174.2	*
Shoot length (mm)	71.7	87.5	69.2	83.3	NS
Root mass (g)	72.9	109.1	34.3	43.7	**
Shoot mass (g)	73.2	125.3	56.7	78.0	*
Root:shoot (lengths)	1.42	1.73	2.34	2.08	NS
Root:shoot (mass)	1.02	0.90	0.62	0.56	**

NS,Not significant. $^{*}P<0.05$, $^{**}P<0.01$

ratios being higher in the <u>Leucadendron</u> spp. than the former (though not significant).

Relative growth rates of the whole seedling (i.e. root and shoot mass combined) with respect to seed mass, were similar in all species (no significant difference, Kruskal-Wallis analysis) (Table 5.5). Root relative growth rates in each of the two <u>Leucadendron</u> spp. were significantly lower than their respective shoot relative growth rates. Relative growth rates of roots and shoots were similar in the <u>Protea</u> spp.

5.4.3 <u>Cone, stem and leaf size</u>

<u>Protea obtusifolia</u> and <u>Leucadendron meridianum</u> had greater cone and leaf mass, and stem diameters than <u>P.susannae</u> and <u>L.coniferum</u> respectively (Table 5.6).

5.5 DISCUSSION

The trend for increased seed size on the colluvial sands - both species having higher mass than their sister taxa on the limestone - can be interpreted as convergent selection for larger seed, thus enabling successful seedling establishment on soils of both lower nutrient status and soil water status. The same argument holds for both factors. Since large seeds produce large seedlings, the enhanced root systems of the latter would enable seedlings to maximise both nutrient and water uptake from the soil. The lower seed mass of both <u>Leucadendron</u> spp. than the <u>Protea</u> species, irrespective of soil type, indicates that a factor other than the soil environment is also operating. This could be a phylogenetic constraint acting on seed size in

Table 5.5. Relative growth rates (g g^{-1} $week^{-1}$) of seedlings and their component roots and shoots of _Protea obtusifolia_, _Leucadendron meridianum_ (limestone) and _P.susannae_, _L.coniferum_ (colluvial sands). Seedlings were grown in root boxes under greenhouse conditions for 16 weeks after germination. Data are mean values. Significance of differences between root and shoot relative growth rates was determined using Wilcoxon's paired-sample test. _n_=6 (_P.susannae_ and _L.coniferum_) and _n_=5 (_P.obtusifolia_ and _L.meridianum_).

	Protea obtusifolia	_Protea susannae_	_Leucadendron meridianum_	_Leucadendron coniferum_
Whole seedling	0.585	0.593	0.600	0.589
Root	0.543	0.546	0.539	0.525
Shoot	0.542	0.553	0.570	0.562
	NS	NS	*	*

NS=Not significant. *$P<0.05$.

Table 5.6. Cone (without seeds), stem and leaf size of _Protea obtusifolia, Leucadendron meridianum_ (limestone) and _P.susannae, L.coniferum_ (colluvial sands). Date are means (SE). _n_=30.

	Protea obtusifolia	_Protea susannae_	_Leucadendron meridianum_	_Leucadendron coniferum_
Cone mass (g)[1]	16.2 (0.7)	9.9 (0.2)	4.5 (0.1)	9.7 (0.3)
Stem diameter (mm)[2]	10.7 (0.2)	8.4 (0.2)	3.0 (0.1)	4.9 (0.2)
Leaf mass (g)[1]	1.18 (0.06)	0.45 (0.03)	0.05 (0.01)	0.10 (0.01)

[1] Dry weight

[2] Measured at base of cone

Leuacdendron spp. A phylogenetic base for adaptional explanations is stressed by Wanntorp _et al_. 1990, and for interpreting the ecological basis of seed size optimization in particular by Hodgson & Mackey (1986) and Mazer (1990). I have found that within genera it appears that environmental selection has acted on seed size. Inter-generic comparisons do not show this same trend. A correlation of increased seed weight with decreasing soil fertility was also found in 12 closely-related grass species of the genus _Chionochloa_ (Lee & Fenner 1989). In contrast, an Australian study showed no differences in seed mass of species occurring on fertile and infertile soil-sites (Westoby, Rice & Howell 1990). The latter study, however, was fertile seeds buffered amongst many infertile seeds on an open receptacle (Coetzee & Giliomee 1987; P.J.Mustart, M.G. Wright & R.M.Cowling, in preparation) from Leucadendron spp. (closed cones, with a woody bract enclosing each seed). Insect predation is higher in P.obtusifolia than P.susannae (higher proportions of predated cones and seed in the former) (Section 6) and I propose that selection for improved protection against insect predation in P.obtusifolia has resulted in high numbers of infertile seed , and hence large infructescences.

Overall, relative growth rates with respect to seed mass were the same in all species, and fall within the range of low relative growth rates reported by Grime & Hunt (1975) for seedlings of "stress-tolerant" plants. A low relative growth rate is believed to allow for sustained growth in a low nutrient regime (Chapin, 1982). The lower Leucadendron root relative growth rates with respect to their shoot rates suggest that this smaller-seeded genus is at a disadvantage in obtaining water and nutrients compared to the large-seeded Protea spp., which have similar root and shoot relative growth rates. Whereas Leucadendron roots weighed less than Protea roots, they were longer. Using mass to determine root:shoot ratios, Leucadendron spp. had lower ratios than the Protea spp. Using lengths the reverse holds. Since the adult Protea - Leucadendron pairs co-exist, I can presume that at this early seedling stage there is no competitive exclusion of either genus, despite the larger root systems of the former. Cody (1986) provides evidence of root niche differentiation in adult desert plant species, suggesting that this mediates their co-existence. It appears that seedlings of these two genera also fill different root niches. The Protea spp. have larger (greater mass), though shorter root systems which enable water and nutrient uptake from the upper soil layers, whilst the Leucadendron spp, have smaller (less mass), though longer root systems in order to reach water and nutrients at greater depths.

The finding that concentrations of phosphorus and nitrogen were similar in the different seed sizes of all four study species agrees with the findings of Esler et al. (1989), but differs from those of Pate et al. (1986) who found a trend of increased nutrient concentration with decreasing Proteaceae seed size. In the species used on this study, and that of Esler et al. (1989), increased seed nutrient contents occurs with increasing seed size. However, unlike the latter study where a trade-off between seed size and number was found, this did not occur here. Mature 17 year-old plants of the smaller-seeded Leucadendron meridianum produce 965 current year seed, whereas similar-aged L.coniferum have correspondingly 9387 current year seeds - a ten-fold difference (Section 6, Table 6.6). Similarly, mature P.obtusifolia plants have an annual seed crop of 819, and P.susannae, with its larger seeds produces 753 seeds, with no indication of a trade-off resulting in decreased numbers. This indicates that seed numbers in these species are not nutrient limited.

In conclusion, I have shown increased seed size in species occurring on a lower nutrient and drier soil environment, and suggest that both soil nutrients and moisture contents could be a selective force determining seed size. This trend was within, but not across genera stressing the importance of considering phylogenetic constraints when investigating adaptations. It would be difficult to test separately for the effects of either nutrients or soil water contents on seed size, since the same arguments hold for both. The increased root:shoot ratios (using length measurements) of the <u>Leucadendron</u> spp. is interpretted as an attempt to overcome the phylogenetically imposed smaller seed size of this species than that found in the <u>Protea</u> spp.

5.6 REFERENCES

Baker, H.G. (1972). Seed weight in relation to environmental conditions in California. Ecology, 53, 997-1010.

Bond, W.J. & Midgley, J. (1988). Allometry and sexual differences in leaf size. American Naturalist, 131, 901-910.

Chapin, F.S. 1982. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics, 2, 233-260.

Cody, M.L.(1986). Roots in plant ecology. Trends in Evolution and Ecology, 1, 76-78.

Coetzee, J.H. & Giliomee, J.H. (1987). Seed predation and survival in the fruit of Protea repens (Proteaceae). South African Journal of Botany, 53, 61-64.

Cowling, R.M. (1990). Diversity components in a species-rich area of the Cape Floristic Region. Journal of Vegetation Science, 1, 699-710.

Cowling, R.M.., Campbell. B.M., Mustart, P.J., McDonald, D.M., Jarman, M.L. & Moll, E.J. (1988). Vegetation classification in a floristically complex area: The Agulhas Plain. South African Journal of Botany, 54, 290-300.

Esler, K. J. , Cowling, R.M., Witkowski, E.T.F. & Mustart, P.J. (1989). Reproductive traits and accumulation of nitrogen and phosphorus during the development of fruits of Protea compacta R.Br. (Calcifuge) and Protea obtusifolia Buek. ex Meisn. (Calcicole). New Phytologist, 112, 109-115.

Fenner, M. (1983). Relationship between seed weight, ash content and seedling growth in twenty-four species of Compositae. New Phytologist, 95, 697-706.

Foster, S.A. (1986). On the adaptive value of large seeds for tropical moist forest trees: a review and synthesis. The Botanical Review, 52, 260-299.

Grime, J.P. & Hunt, R. (1975). Relative growth rate: its range and adaptive significance in a local flora. Journal of Ecology, 63, 393-421.

Haig, D. (1989). Seed size and adaptation. Trends in Ecology and Evolution ,4, 145.

Harper, J.L. (1977). Population Biology of Plants. Academic Press, London.

Harper J.L., Lovell, P.H. & Moore, K.G. (1970). The shapes and sizes of seeds. Annual Review of Ecology and Systematics, 1, 327-356.

Hodgson, J.G. & Mackey, J.M.L. (1986). The ecological specialisation of dicotyledonous families within a local flora: Some factors constraining optimization of seed size and their possible evolutionary significance. New Phytologist, 104, 497-515.

Hunt, R. (1978). Plant Growth Analysis. Studies in Biology no. 96. Edward Arnold, London.

Jackson, M.L. (1958). Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, N.J.

Kuo, J., Hocking, P.J. & Pate, J.S. (1982). Nutrient reserves in seeds of selected Proteaceous species from south-Western Australia. Australian Journal of Botany, 30, 231-249.

Lamont, B.B., Collins, B.G. & Cowling, R.M. (1985). Reproductive biology of the Proteaceae in Australia and South Africa. Proceedings of the Ecological Society of Australia, 51, 213-224.

Lee, W.G. & Fenner, M. (1989). Mineral nutrient allocation in seeds and shoots of twelve Chionochloa species in relation to soil fertility. Journal of Ecology, 77, 704-716.

Le Maitre D.C. & Midgley, J.J. (in press). Allometric relationships between leaf and inflorescence mass in the genus Protea (Proteaceae): an analysis of the exceptions to the rule. Functional Ecology.

Mazer, S.J. (1990). Seed mass of Indiana Dune genera and families: taxonomic and ecological correlates. Evolutionary Ecology, 4, 326-357.

Murphy, J. Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimicia Acta, 27, 31-36.

Pate, J.S., Rasins, E., Rullo,J & Kuo, J. (1986). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. Annals of Botany, 57, 747-770.

Rourke, J.P. (1980). The Proteas of Southern Africa. Purnell, Cape Town.

Salisbury, E. (1974). Seed size and mass in relation to environment. Proceedings of the Royal Society of London, 186, 83-88.

Silvertown, J.L. (1989). The paradox of seed size and adaptation. Trends in Ecology and Evolution, 4, 24-26.

Smith, V.R. (1980). A phenol-hypochlorite determination of ammonium-nitrogen in Kjeldhal digests of plant tissue.

Communications in Soil Science and Plant Analysis 14, 925-936.

Stock, W.D., Pate, J.S. & Delfs, J. (1990). Influence of seed size and quality on seedling development under low nutrient conditions in five Australian and South African members of the Proteaceae. Journal of Ecology, 78, 1005-1020.

Stock, W.D., Pate, J.S. Kuo, J. & Hansen, A.P. (1989). Resource control of seed set in Banksia laricina G. Gardner (Proteaceae). Functional Ecology, 4, 453-460.

Thompson, K. (1987). Seeds and seed banks. New Phytologist, 106 (Suppl.), 23-24.

Thompson, K. & Rabinowitz, D. (1989). Do big plants have big seeds? American Naturalist, 133, 722-728.

Thwaites, R.N. & Cowling, R.M. (1988). Vegetation relationships on the Agulhas Plain, South Africa. Catena, 15, 333-345.

Wanntorp, H., Brooks, D.R., Nilsson, T., Nylin, S., Ronquist, F., Stearns, S. & Wedell, N. (1990). Phylogenetic approaches in ecology. Oikos, 57, 119-132.

Westoby, M, Rice,B & Howell, J. (1990). Seed size and plant growth form as factors in dispersal spectra. Ecology, 71, 1307-1315.

Werner, P.A. & Platt, W.J. (1976). Ecological relationships of co-occurring Goldenrods (Solidago : Compositae). American Naturalist, 110, 959-971.

Williams, I.J.M. (1972). A revision of the genus Leucadendron (Proteaceae). Contribution of the Bolus Herbarium No.3.

Wulff, R.D. (1986). Seed size variation in Desmodium paniculatum II. Effects on seedling growth and physiological performance. Journal of Ecology, 74, 99-114.

6.0 ADULT REPRODUCTIVE TRAITS ON DIFFERENT SOIL TYPES

6.1 Abstract

6.2 Introduction

6.3 Materials and methods

  6.3.1 Study site and study species

  6.3.2 Soil analyses

  6.3.3 Structural characteristics

  6.3.4 Patterns of cone and seed production

  6.3.5 Statistical analyses

6.4 Results

  6.4.1 Soil analyses

  6.4.2 Stand characteristics

  6.4.3 Cone and seed production

6.5 Discussion

  6.5.1 Soil regimes

  6.5.2 Parallel trends within both species-pairs

  6.5.3 Non-parallel trends within species-pairs

  6.5.4 Conclusions

6.6 References

6.1 ABSTRACT

High levels of edaphic endemism and soil-related beta-diversity in Agulhas Plain fynbos communities led to the idea that reproductive traits of plants growing on these generally nutrient-poor fynbos soils would be related to differences in nutrient regime. Two closely related Proteaceae species-pairs growing on adjacent, different soil types were investigated for differences in reproductive traits. The species were Protea obtusifolia and Leucadendron meridianum occurring in shallow pockets of limestone-derived soils (higher nutrient concentrations); and P.susannae and L.coniferum on the adjacent, uniformly deep colluvial sands (lower nutrient concentrations). The results showed that species growing on the limestone soil were smaller, with fewer cones and seed per plant, than species on the colluvial sands. This suggests that the small soil pockets of limestone soil limit plant size, in turn limiting the number of reproductive structures. Annual variation in reproductive output was the same in all species. The higher cone and seed predation levels of both limestone species than the colluvial sands species were ascribed to the higher plant densities of the former leading to lower insect search times. There were no consistent trends in degree of serotiny, or female/male function. It was concluded that there were no overall patterns in reproductive traits that could be ascribed to differences in soil regimes, and that these fynbos species had evolved traits sustainable by the nutrient regimes of their soil types, but not directly related to them other than through

size-related effects. Fire regime has almost certainly played a more important role than nutrients in determining reproductive traits.

6.2 INTRODUCTION

Since plants are unable to move in order to acquire resources, they respond or adapt to the environment by developing different patterns of vegetative and reproductive growth (Silvertown & Rabinowitz 1985). Resources are partitioned within individual plants, and it is believed that reproduction, growth and defense compete for limited resources (Bazzaz _et al_. 1987, Chapin _et al_. 1987). This concept would be relevant for South African fire-disturbed heathlands (fynbos) growing on nutrient-poor soils (Taylor 1978), and particularly relevant for Proteaceae which concentrate nutrients in their seeds (van Staden & Brown 1977, Pate _et al_. 1985, Jongens-Roberts & Mitchell 1986). For example, the lower seed set of Proteaceae in Australia than in South Africa has been attributed to the lower soil nutrients and moisture levels in the former region (Lamont, Collins & Cowling 1985).

The Agulhas Plain lowland fynbos communities have high levels of edaphic endemism and species turnover along soil fertility gradients (Cowling 1990). The distinct distribution of co-occurring dominant fynbos species _Protea obtusifolia_ and _Leucadendron meridianum_ on limestone derived soils, and _P.susannae_ and _L.coniferum_ on adjacent, colluvial sands led to the idea of investigating the relationship between soil-type and reproductive traits. The higher nutrient and water concentrations of the limestone soil than the colluvial sands has been shown to have consequences on seed size, and seedling emergence and establishment patterns in these species (Sections 2,3,5). However, adult plant requirements differ from those of these early stages. Since the limestone soils occur in shallow pockets (0-30 cm) in the limestone bedrock, in contrast to the uniformly deep (>1 m) colluvial sands, it is likely that the differences in concentration would be offset by differences in the absolute amounts of soil resources. This is likely to make the former habitat more resource-limiting than the latter to large, woody adult plants.

Since the two _Protea_ spp. and _Leacadendron_ spp. are both closely related species-pairs, this provides an opportunity to study the effect of the soil regime on plant reproductive traits, without the constraints of phylogeny (Wanntorp _et_ _al_. 1990). A trend occuring between the two species of each genus can be attributed to an environmental association, and no taxonomic explanation need be invoked. Since the species-pairs occur on adjacent habitats, the effects of mesoclimate and fire regimes are similar for all species.

Selected reproductive traits of these species were quantified. Parallel reproductive trends within each of the two species-pairs (i.e. across soil types) would indicate a response to different edaphic environments. Non-parallel trends between species-pairs would indicate a response to some other factor. The following reproductive traits were investigated: juvenile period, annual outputs of cone and seed, number of seed per cone and seeds per plant, and degree of serotiny. Female to male function (female:male plant ratios in the dioecious _Leucadendron_ spp., and fertile seed:infertile seed ratios in the hermaphrodite _Protea_ spp.) was also investigated.

6.3 MATERIALS AND METHODS

6.3.1 Study site and study species

The study site was situated in the coastal lowlands of the Agulhas Plain (34° 35' S, 19° 55' E) in the south-western fynbos biome (Moll et al. 1984). The region has a mediterranean climate. The mean annual rainfall is 452 mm of which 65% falls between April and September. The soils are derived from the Mio-Pliocene limestone of the Bredasdorp Formation (Thwaites & Cowling 1988). Whereas no soil moisture determinations were performed at the study site, measurements obtained from similar soil types on the Agulhas Plain showed that throughout the year soil surface (2 to 15 cm) moisture contents were higher in limestone soils (October to March: 3 to 11% ; April to September: 7 to 16%) than colluvial sands (<1 to 2% ; and 2% respectively) (see Section 2).

The Protea spp. are hermaphroditic and bear seeds on an open, slightly convex receptacle. The Leucadendron spp. are dioecious and female plants have seed borne in cones. All are killed by fire and regenerate solely from seed. They are overstorey dominant shrubs in the Proteoid Fynbos vegetation (Cowling et al. 1988).

6.3.2 Soil analyses

Five randomly chosen samples were taken from the top 100 mm of soil in both habitats. Analyses of soil pH, texture and nutrients were performed by the Soil Science Section of the

Winter Rainfall Region, Department of Agriculture. A perchloric acid extract was read on a Beckman DC Emission spectrophotometer to give concentrations of total phosphorus, exchangeable cations (Na, K, Ca and Mg), iron and aluminium. Oxidizable carbon gave a measure of organic carbon and nitrogen was determined by Kjeldahl digestion. Bulk density was determined by weighing a known volume of soil after passing through a 2 mm sieve.

6.3.3 Structural characteristics

In each habitat the number of individuals of each species was counted (in the Leucadendron spp. the sex was noted) in ten randomly chosen 10 x10 m^2 plots. Height and canopy diameter were measured. Canopy volume was calculated as the volume of an ellipsoid ($4/3.pi.r_1.(r_2)^2$, where r_1=height/2; r_2=diameter/2).

6.3.4 Patterns of cone and seed production .

During March 1987, 30 individuals of each species were randomly chosen. Cones (mature infructescences) were aged by node counts (Bond 1985). The number of cones in the age classes 1, 2, 3 and 4 years and older for the Protea spp.,and 1, 2, 3, 4 and 5 years and older for the Leucadendron spp., were counted. Since node counting beyond 4 to 5 years was difficult, numbers of cones in these older categories were lumped. Five cones per age class were removed from each individual for seed analysis and were oven-dried at 40°C for 48 hours to facilitate seed release. Cones were examined for evidence of insect predation (frass or holes). Seeds were counted after sorting by feel and weighing into fertile (fertile, embryo-filled seeds felt plumper, and were heavier than infertile seeds), infertile and predated categories. The age of first reproduction was determined by node counting to the earliest appearance of cones.

6.3.5 Statistical analyses

Differences in cone and seed numbers, according to species within genera; species within soil type; and species themselves, was assessed by two-way analysis of variance of the square root of data. When zero values were present in a data set, 0.5 was added prior to square root transformation (Zar 1984). Annual variation in cone and seed numbers fits a random blocked design (cones or seeds were counted from different years on the same plant), and to eliminate individual plant variability two-way analysis was performed. In seed number analyses, the mean of five values was used (five cones per individual per year were sampled).

Significance of the correlation between annual numbers of cones or seeds with time was assessed by Spearmans rank correlation. Two-way chi-squared analysis of frequencies was performed to establish differences between frequencies of female and male Leucadendron plants, and between frequencies of fertile and infertile seed in the Protea spp.. Statgraphics software (Statistical Graphics Corporation, Inc.) was used for all analyses.

6.4 RESULTS

6.4.1 Soil analyses:

Whilst both soil types consisted of more than 90 percent sand, the limestone soils contained a higher proportion of finer particles than the coarser deep sands (Table 6.1). The latter soil had greater bulk density (1.4 g/cm^3) than the former (1.1 g/cm^3). The limestone soils were more alkaline, and had higher concentrations of carbon, nitrogen and calcium. Phosphorus levels were similar in both soil types. However, values obtained from independent analyses of similar soil types supporting the same Proteaceae spp. in the same and separate areas in the Agulhas Plain showed that limestone soils had on the whole higher total phosphorus concentrations, as well as more pronounced differences in pH and carbon, nitrogen and calcium concentrations (Table 6.2).

6.4.2 Stand characteristics:

At the time of sampling, populations of all species were 17-18 years old (time since last fire). Age of first reproduction was at about 7-8 years for all species.

The limestone species, Protea obtusifolia and Leucadendron meridianum, were shorter, with smaller canopy volumes, and occurred at higher densities than the colluvial sands species, P.susannae and L.coniferum (Table 6.3). There was no significant difference between the frequencies of female and male plants of each of L.coniferum and L.meridianum. Whilst plant height did

Table 6.1. Topsoil characteristics of Proteoid Fynbos communities at the study site on the Agulhas Plain. Data are means (SE). *n*=5

Dominant proteoid shrubs	*Protea obtusifolia*/ *Leucadendron meridianum*	*Protea susannae*/ *Leucadendron coniferum*
Soil type	Shallow sand on limestone	Deep colluvial sands
Clay (%)	0.0	0.0
Silt (%)	4.6 (1.0)	3.4(0.6)
Fine sand (%)	61.4 (4.4)	49.2 (3.8)
Medium sand (%)	33.0 (4.2)	42.4 (4.3)
Coarse sand (%)	1.0 (0)	5.0 (0.7)
pH	6.3 (0.1)	5.3 (0.1)
Total phosphorus (mg/kg)	45.5 (5.7)	53.0 (3.4)
Organic carbon (%)	3.2 (0.4)	2.1 (0.2)
Nitrogen (%)	0.09 (0.01)	0.06 (0.005)
Sodium (mg/kg)	34.5 (6.9)	25.3 (2.3)
Potassium (mg/kg)	39.1 (1.6)	46.9 (3.5)
Calcium (mg/kg)	1660.0 (230.0)	684.0 (40.0)
Magnesium (mg/kg)	79.3 (11.0)	68.3 (3.7)
Iron (mg/kg)	1508 (217)	1503 (150)
Aluminium (mg/kg)	2356 (158)	2545 (220)

Table 6.2. Soil characteristics of Proteoid Fynbos communities at different areas in the Agulhas Plain. Data are means and were compiled from Witkowski & Mitchell (1987), Thwaites & Cowling (1988), Esler _et_ _al_. (1989) and P.J.Mustart & R.M.Cowling, unpublished data. Significance of differences between communities was determined using the Mann-Whitney _U_ test.

Dominant proteoid shrubs	_Protea obtusifolia/ Leucadendron meridianum_		_Protea susannae/ Leucadendron coniferum_	
Soil type	Shallow soil on limestone		Deep colluvial sands	
	Mean	Number of sites	Mean	Number of sites
pH	7.2	8	5.6*	3
Total phosphorus (mg/kg)	157.5	4	33.0#	2
Organic carbon (%)	5.5	8	1.6*	4
Nitrogen (%)	0.24	8	0.05***	4
Calcium (mg/kg)	1960.0	6	580.0*	3

*$P<0.05$, ***$P<0.001$, NS=Not significant

#Insufficient replicates for statistical analysis.

Table 6.3. Selected structural characteristics of *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum* plants at the study site. Data are means (SE). Significance of differences between species of stand density (*n*=10), plant height and canopy volume (*n*=30), was obtained by one - way analysis of variance. Different letters (a,b,c,d) indicate significance according to Tukey's multiple range test.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*	
a. Stand density (plants per 100 m^2)					
	8.5 (1.6)^{ab}	19.4 (2.0)^{c}	4.5 (1.6)^{a} 2.0 (0.6)$^{\#}$	13.8 (2.4)^{bc} 6.5 (1.4)$^{\#}$	***
b. Plant height (m)					
	2.4 (0.04)^{c}	1.3 (0.02)^{a}	2.5 (0.05)$^{c,\#}$	1.7 (0.03)$^{b,\#}$	***
c. Canopy volume (m^3)					
	14.7 (1.6)^{c}	2.7 (0.2)^{a}	9.9 (0.9)$^{b,\#}$	2.0 (0.2)$^{a,\#}$	***

***$P<0.001$, NS=Not significant

#Female plant data

not vary much within each species (coefficients of variation for each species ranged from 9.7% to 10.2%), there was greater variation in canopy volumes (coefficients of variation ranged from 47.9% to 60.2%).

6.4.3 Cone and seed production:

i. Genus and soil type patterns (Table 6.4).

Overall generic or soil type trends of cone and seed production in the two-way analysis of variance showed that when species were combined into genera there were significantly higher total cone, and total fertile seed numbers, but lower predated seed, per plant in the (female) Leucadendron individuals than in those of the Protea individuals. Similarly, combined colluvial sands species (P.susannae with L.coniferum) had significantly more cones, and fertile seed per plant than the combined limestone soil species (P.obtusifolia and L.meridianum). There was no difference in total predated seed numbers per plant between species from the two soil types.

The differences in cone production were consistent (no significant interaction between genus and soil type), with L.coniferum producing 2.3 times more cones per plant than L.meridianum, and P.susannae 2.7 times more cones than P.obtusifolia. Similarly individuals of L.coniferum had 3.2 times more cones than those of P.susannae, and individuals of L.meridianum 3.9 times those of P.obtusifolia. However, L.coniferum had disproportionately more fertile seed per plant than L. meridianum (9x) and P.susannae (8x), while P.susannae

Table 6.4. Differences in a) total cones per plant, b) total fertile seed per plant, and c) total predated seed per plant between genera (combined *Protea* spp. and combined (female) *Leucadendron* spp.); soil types (*P.susannae* and *L.coniferum* combined, *P.obtusifolia* and *L.meridianum* combined); and interactions between genus and soil type, by two-way analysis of variance on the square root transformations of data. Data are means (SE). *n* = 30 plants per species. Cs = Colluvial sands. Li = Limestone soil.

Genus		Soil type		Genus x soil type	
a. Total cones per plant					
Protea	172.5 (19.0)	Cs	535.5 (58.4)	*P.susannae*	252.6 (31.5)
Leucadendron	590.8 (55.4)	Li	227.9 (26.7)	*P.obtusifolia*	92.4 (6.6)
				L.coniferum	818.3 (85.8)
				L.meridianum	363.4 (39.9)
F	112.5 ***		55.5 ***		3.2 NS
b. Total fertile seed per plant					
Protea	2390.0 (304)	Cs	13601.0 (2116)	*P.susannae*	3178.0 (523)
Leucadendron	13287.0 (2136)	Li	2076.0 (235)	*P.obtusifolia*	1602.0 (242)
				L.coniferum	24024.0 (3234)
				L.meridianum	2550.0 (389)
F	82.2 ***		96.2 ***		54.2 ***
c. Total predated seed per plant					
Protea	3710.0 (384.0)	Cs	2004.0 (368.0)	*P.susannae*	3634.0 (604.0)
Leucadendron	412.0 (47.0)	Li	2118.0 (326.0)	*P.obtusifolia*	3784.0 (486.0)
				L.coniferum	373.0 (62.0)
				L.meridianum	451.0 (71.0)
F	130.5 ***		0.5 NS		0 NS

***P<0.001, NS=Not significant.

plant seed numbers were only twice that of P.obtusifolia, and L.meridianum individuals 1.5 times those of P.obtusifolia (significant genus x soil type interaction).

Whereas both Protea susannae and P.obtusifolia had similar numbers of predated seed per plant, as did Leucadendron coniferum and L.meridianum, when predated seed numbers were related to fertile seed numbers, different patterns were evident. Protea species had higher predated:fertile seed ratios than Leucadendron species, and, within genera, the limestone species had higher ratios than their colluvial sands counterparts (P.susannae 1.2 ;P.obtusifolia 2.4 ; L.coniferum 0.02 ; L.meridianum 0.18). A similar trend was found if predated seed was expressed as a percentage of total seed (fertile + infertile + predated) as follows:- P.susannae 15.9%; P.obtusifolia 28.8%; L.coniferum 1.4%;and L.meridianum 7.2%.

ii. Annual variability.

In all species, annual numbers of cones, numbers of fertile seeds per cone, and total numbers of fertile seed varied significantly (Table 6.5). Numbers of predated seeds per cone, and total predated seed numbers also showed significant variation, with the exception of Leucadendron coniferum where no significant annual variation occurred. Comparing F values with similar degrees of freedom showed that annual numbers of cones, seeds per cone, and total fertile seed per plant, varied more in L.coniferum than L.meridianum. Not shown in the table was the significant individual plant variation for all species, in each category (a to e).

Table 6.5. *F* values obtained by two-way analysis of variance (random blocked design of cone and seed numbers counted from different years on the same plant enabled individual plant variability to be eliminated, see 6.3.5) of annual numbers of a) cones, b) fertile seeds per cone, c) total fertile seed, d) predated seeds per cone, and e) total predated seed over four years for *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum*. *n*=30. Data used were square root transformations.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
a. Cones per year				
	*** 6.5$^{\#}$	*** 7.4$^{\#}$	***28.8$^{\#}$	*** 7.7$^{\#}$
b. Fertile seed per cone per year				
	***14.2$^{\$}$	***25.5^{+}	***38.4$^{\#}$	***19.3$^{\#}$
c. Total fertile seed per year				
	***13.7$^{\$}$	***19.1^{+}	***50.9$^{\#}$	***17.8$^{\#}$
d. Predated seed per cone per year				
	***19.4$^{\$}$	***10.0^{+}	NS 2.6$^{\#}$	** 5.8$^{\#}$
e. Total predated seed per year				
	*** 8.1$^{\$}$	** 5.4^{+}	NS 1.8$^{\#}$	* 4.0$^{\#}$

*P<0.05, **P<0.01, ***P<0.001, NS=Not significant.

$^{\#}$Df=3,87; $^{\$}$Df=3,83; $^{+}$Df=3,61.

The coefficients of variation for annual cone production (years 1 - 4) was; Protea susannae 85.7% ; P.obtusifolia 60.2%; L.coniferum 93.3% ; and L.meridianum 71.3%.

iii. Contributions from years of increasing cone age.

During the four years prior to this study, Leucadendron coniferum had increasing numbers of cones in each successive year (significant negative correlation of cone number and cone age, Table 6.6). In the other species the correlations were either low or non-significant. On the other hand, in all species except Protea susannae which had relatively low numbers of seeds from the most recent cones , there was a significant decrease in the contribution of fertile seed from cones of increasing age.

Predated seed numbers increased significantly in Protea susannae with increasing cone age (Table 6.6). P.obtusifolia predated seed numbers were generally high in all age classes showing no significant increase wih increasing cone age. There were low annual numbers of predated seed in both Leucadendron coniferum (no correlation with cone age) and L.meridianum (low correlation with cone age). These low values are not likely to reflect real predation levels since older cones of Leucadendron species, especially those bored into by insect predators, open and release their seed (pers. observation) thus precluding the quantification of predated seed numbers. The flat receptacles of the Protea species retain seed in older cones allowing a more accurate indication of seed predation levels.

The proportion of predated cones also had a positive correlation with cone age (Figure 6.1) in all species except

Table 6.6. Annual contributions of a) cones , and b) total fertile seed , and c) fertile seed as a percent of the total seed bank of: *Protea susannae*, *P.obtusifolia*, *Leucadendron coniferum* and *L.meridianum*. Data are mean (SE). *n* = 30 individuals. *r* = Spearman's rank correlation with cones age (1 - 4 or 5 years).

Cone age (years)	*Protea susannae*	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
a. Cones per year				
1	35.7 (6.9)	19.0 (1.8)	220.0 (31.5)	74.0 (11.4)
2	46.7 (6.3)	18.2 (1.8)	203.8 (32.6)	58.9 (6.7)
3	46.5 (6.7)	11.2 (1.4)	118.5 (13.3)	61.6 (7.3)
4	29.6 (4.2)	15.6 (1.7)	73.2 (9.2)	51.0 (5.1)
5	31.1 (4.8)	9.9 (1.3)	35.4 (4.7)	42.8 (4.3)
r	0.003 NS	-0.2 *	-0.48***	-0.13 NS
b. Fertile seed per year				
1	753 (181)	819 (110)	9387 (1337)	965 (190)
2	1162 (219)	570 (143)	8446 (1367)	643 (115)
3	996 (162)	342 (99)	4091 (536)	562 (91)
4	367 (67)	178 (52)	1775 (329)	244 (44)
5	-	-	324 (75)	135 (36)
r	-0.12 NS	-0.58***	-0.59***	-0.4***
c. Predated seed per year				
1	492 (151)	820 (132)	75 (26)	69 (20.5)
2	929 (223)	1617 (198)	130 (32)	144 (26.9)
3	887 (197)	1079 (142)	79 (18)	121 (22.4)
4	1438 (256)	1460 (323)	65 (16)	102 (27.7)
5	-	-	22 (7)	139 (4.8)
r	0.33***	0.14 NS	-0.02 NS	-0.06 NS
d. Annual fertile seed as a percent of total seed bank (%).				
1	21.8	50.8	40.0	38.4
2	33.9	28.4	35.0	25.2
3	33.0	13.0	17.0	21.3
>3	11.3	7.8	8.0	15.1

* P<0.05, **P<0.01,***P<0.001. NS = Not significant

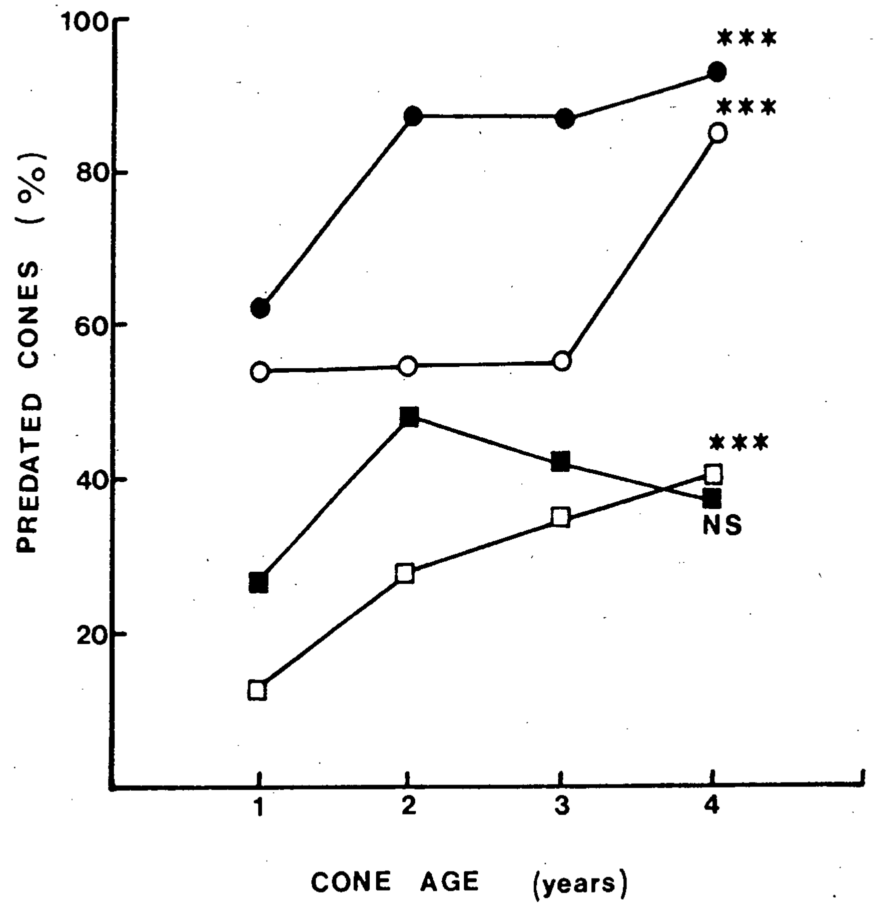


Figure 6.1. Percent predated cones (cones containing predated seed, or evidence of predation such as frass and insect holes) stored on the canopy for increasing times, of Protea susannae (o), P.obtusifolia (●), Leucadendron coniferum (□) and L.meridianum (■). n=30 plants per species (up to 5 cones per age class). ***$P<0.001$, NS=Not significant, using Spearmans rank correlation of percent predated cones with cones age.

Leucadendron meridianum which showed a trend reversal in years three and four. This was most likely due to the large number of empty cones with no apparent signs of predation. In all age categories there were higher percentages of predated cones in Protea obtusifolia than P.susannae. Similarly L.meridianum had higher percentages than L.coniferum, except in the 4 year-old category where it was slightly higher in the latter species.

iv. Serotiny.

Strongly serotinous species have relatively low proportions of the total seed bank contributed by the most recent crop. Protea susannae was the most serotinous having only 21.8% of the total seed crop in current year cones (Table 6.6). P.obtusifolia, the least serotinous, had 50% of the seed bank in the most recent cones. Both Leucadendron species had intermediate values of about 40%. In all species the contribution from cones of three years and older was 15 percent or less.

v. Seed set per cone.

Seed set per cone was lower in the Protea species than in the Leucadendron species (Table 6.7). There was a significant difference between the frequencies of numbers of fertile seed (successful female function) and infertile seed (unsuccessful female function) between P.susannae and P.obtusifolia, with the latter having a higher female to male frequency than the former ($P<0.001$; two-way chi-squared analysis of frequency).

Table 6.7. Seed set per cone in _Protea susannae_, _P.obtusifolia_, _Leucadendron coniferum_ and _L.meridianum_.

	Protea susannae	_Protea obtusifolia_	_Leucadendron coniferum_	_Leucadendron meridianum_
Seed set (%)[#]	12.7	19.5	87.1	54.7
CV (%)[$]	59.4	62.1	13.3	52.9
Number of cones analysed	54	52	130	119

[#] Mean values (fertile seeds expressed as a percentage of total fertile plus infertile seeds) calculated from year one unpredated cones.

[$] Coefficient of variation of percent seed set per cone.

6.5 DISCUSSION

6.5.1 Soil regimes

Proteaceae have dense root clusters (proteoid roots) concentrated in the top 10 cm of soil, maximising nutrient uptake during the wet season (Lamont 1982). It can thus be assumed that topsoil nutrient characteristics only are needed in assessing the ecological importance to the study species.

Apart from calcium, there is very little difference in nutrient concentrations between the limestone soils and the colluvial sands at the study site. The slightly higher nitrogen and carbon concentrations of the former than the latter would, in terms of soil volume availabilty, be offset by the higher bulk density of the latter. These nutrient levels differ from independent analyses of both the same and similar soil types in separate areas where limestone soils have greater concentrations of phosphorus, carbon and nitrogen than colluvial sands (Table 6.2).

Despite these discrepancies, the basic difference in soil volume would be overriding, making the uniformly deep colluvial sands potentially less nutrient limiting than the smaller pockets of limestone soil.

Soil moisture regimes are more difficult to define. The surface moisture determinations might be of no relevance to plants with deep roots. Proteaceae roots on the limestone can penetrate cracks for several metres (pers. observation). Mid-summer pre-dawn water potentials, an indication of the soil water status, were similar in all four study species (unpublished data).

6.5.2 Parallel trends within both species-pairs.

Both limestone species were shorter, and had smaller canopy volumes than the colluvial sand species. These are most likely related to the volume of soil available. Protea obtusifolia when occasionally found growing on the colluvial sands is larger than counterparts on the limestone (pers observation). A relation between soil depth and plant height has also been suggested for the South African Proteaceae by Midgley (1986).

All species reached reproductive maturity at 7-8 years. There was with no delayed reproduction which would enable limited resources to be used at earlier stages for vegetative growth, in time leading to increased size-related reproductive effort (Begon, Harper & Townsend 1986). The similar juvenile periods of all species is likely to have been determined by a fire regime where frequencies occurred at intervals greater than this period (Gill & Groves 1981).

There were also parallel differences within genera in numbers of cones and seed per individual. The colluvial sands species, P.susannae and L.coniferum, had more cones and fertile seed per plant than P.obtusifolia and L.meridianum respectively. These reproductive outputs were, in the main, size related, stressing the importance of relationships between reproductive effort and plant size (Samson & Werk 1986). Leucadendron coniferum stands out in having disproportionately more seed than

P.susannae despite having a smaller mean canopy volume. There is likely to be a demographic explanation for this (see Section 8.2). It appears that concentrations of soil nutrients do not limit cone or seed numbers. It is suggested that smaller plants on the limestone soils produce fewer cones and seeds as function of size, than their matched taxa on the colluvial sands.

Annual variation of reproductive effort on either soil type was similar in all species, with significant variation of cone numbers, fertile seed per cone, and total fertile seed numbers. Of all the species the greatest variability of cones and total fertile seed per year occurred in Leucadendron coniferum. This was largely due to a general increase with time - the only species to have a highly significant correlation of cone numbers with decreasing cone age - i.e. increasing plant age - rather than annual variability.

There were soil-related trends trends in predation levels within both genera. The limestone species, P. obtusifolia and L.meridianum had higher predation levels (percentage predated cones, and seed, as well as predated seed : fertile seed ratios) than their respective colluvial sands generic counterparts. These higher predation levels were not related to higher plant seed numbers (a reliable food source) of the former than the latter. Both limestone species have smaller seeds than P.susannae and L.coniferum respectively, and all have similar nutrient concentrations (Section 5, Table 5.3). Why there are these differences is not known. There is a relation between predation levels and variation of cone production. Within genera, the coefficient of variation of cone numbers of the limestone species was lower than the colluvial species (Protea susannae 85.7% ; P.obtusifolia 60.2% ; L.coniferum 93.3% ; and L.meridianum 71.3%). It is possible that both the higher predictability of food supply resulting from this lower variability, and the higher stand densities of the limestone plants, would lead to decreased insect search time (Root 1973, Forcella 1980), thus increasing the likelihood of predation.

6.5.3 Non-parallel trends within species-pairs

There was no consistent trend in degree of serotiny in the two genera. Protea susannae had a lower proportion of seeds in current year cones (21.8%) than P.obtusifolia (50.8%). The corresponding proportions in the Leucadendron spp. were similar (40.0%, 38.4%). Serotiny has been suggested to be a mechanism dampening the fluctuations in reproductive output (Lamont et al. 1991), but I found no relationship between variability of cone or fertile seed production and degree of serotiny. A similar conclusion was reached by Cowling et al. (1986) for Australian Banksia spp. (Proteaceae).

There was also no consistent trend in female/male function. There were similar frequencies of female and male plants of the dioecious Leucadendron spp. on both soil types. If female reproductive effort is resource limited (Goldman & Willson 1986, Sutherland 1986, Devin 1988), then these results indicate neither soil habitat to be limiting. On the other hand P.obtusifolia had higher female (fertile seed numbers) to male (infertile seed numbers) function than P.susannae, suggesting a relation between high soil nutrient concentrations and increased female function. This is, however, based on the assumption that infertile seeds are functionally andromonoecious (Bawa and Beach 1981, Rebelo & Rourke 1986). Since there are alternate explanations for the large infertile seed component in Protea spp. (flower or seed predation, pollen limitation, genetically based mortality and predator avoidance (Ayre & Whelan 1989)), a resource-controlled explanation would not have much weight in this context.

6.5.4 Conclusion

This study shows no clear, overall pattern in reproductive traits, other than size-related effects, that can be ascribed to differences in soil regimes. Plants growing on infertile soils have low growth rates and luxury consumption of nutrients (plant-tissue accumulation of nutrients during pulses of availability, for re-distribution within the plant at times of low nutrient availability) (Chapin 1982). This implies an overall plant adaptation to low soil nutrients, and could apply to fynbos species. Despite the differences in nutrient concentrations in these soil types, they are both low relative to non-fynbos soil types (Witkowski & Mitchell 1987, Thwaites & Cowling 1988). It appears that these fynbos Proteaceae have evolved reproductive traits sustainable by the nutrient regimes of their soil types, yet not directly related to them, other than through size-related effects.

The recurrent fires to which these obligate reseeding fynbos species are subjected are likely to have played a major role in determining reproductive traits that will maximise the post-fire perpetuation of each species (Gill 1981, Lamont et al. 1991). It is possible that adaptations to achieve this purpose are overriding to any soil nutrient determined reproductive trait.

6.6 REFERENCES

Ayre, D.J. & Whelan R.J. (1989). Factors controlling fruit set in hermaphroditic plants: studies with Australian Proteaceae. Trends in Ecology and Evolution, 4, 267-272.

Bawa, K.S. & Beach, J.H. (1981). Evolution of sexual systems in flowering plants . Annals of the Missouri Botanical Gardens, 68, 254 - 274.

Bazzaz, F.A., Chiariello, N.R., Coley, P.D. & Pitelka, L.F. (1987). Allocating resources to reproduction and defense. Bioscience, 37, 58 - 67.

Begon, M., Harper, J.L. & Townsend, C.R. (1986). Ecology. Individuals, Populations and Communities. Blackwell Scientific Publications.

Bond, W. J. (1985). Canopy stored seed reserves (serotiny) in Cape Proteaceae. South African Journal of Botany, 51, 181-186.

Chapin, F.S., 111 (1982). The mineral nutrition of wild plants. Annual Review of Ecology and Systematics, 11, 233-260.

Chapin, F.S., 111, Bloom, A.J., Field, C.B. & Waring, R.H. (1987). Plant responses to multiple environmental factors. Bioscience, 37, 49 - 57.

Cowling, R.M. (1990). Diversity components in a species-rich area of the Cape Floristic Region. Journal of Vegetation Science, 1, 699-710.

Cowling, R.M., Campbell , B.M., Mustart, P.J., McDonald, D.M., Jarman, M.L. & Moll, E.J. (1988). Vegetation classification in a floristically complex area: The Agulhas Plain. South African Journal of Botany, 54, 290 - 300.

Cowling, R.M., Lamont, B.B. & Pierce, S.M. (1986). Seed bank dynamics of four co-occurring Banksia species. Journal of Ecology, 75, 289 - 302.

Devin, B. (1988). The effects of stress on characters of Lobelia cardinalis. Ecology, 69, 1716-1720.

Forcella, F. (1980). Cone predation by pinyon pine beetle (Conophorus edulis; Scolytidae): Dependence on frequency and magnitude of cone production. American Naturalist 116, 594-598.

Gill, A.M. (1981). Adaptive responses of Australian vascular plants species to fires. From: Gill, A.M., Groves, R.H.C., Noble, I.R. (Eds) Fire and the Australian Biota. Australian Academy of Science, Canberra.

Gill, A.M. & Groves, R.H. 1981. Fire regimes in heathlands and their plant-ecological aspects. From: Specht, R.L. (Ed.) Heathlands and Related Shrublands of the World, B. Analytical Studies.. Elsevier Scientific Publishing Company, Amsterdam.

Goldman, D.A. & Willson, M.F. (1986). Sex allocation in hermaphroditic plants: a review and critique. The Botanical Review, 52, 157 - 194.

Jongens-Roberts, S.M. & Mitchell, D.T. (1986). The distribution of dry mass in an evergreen fynbos shrub species Leucospermum parile (Salisbury ex J. Knight) Sweet (Proteaceae), at different stages of development. New Phytologist, 103, 669-683.

Lamont, B. (1982). Mechanisms for enhancing nutrient uptake in plants, with particular reference to mediterranean South Africa and western Australia. The Botanical Review, 48, 597- 689.

Lamont, B.B, Collins, B.G. & Cowling, R.M. (1985). Reproductive biology of the Proteaceae in Australia and South Africa. Proceedings of the Ecological Society of Australia, 14, 213-224.

Lamont, B.B., Le Maitre, D.C., Cowling, R.M. & Enright, N.J. (1991). Canopy seed storage in woody plants. Botanical Review.

Midgley, J.J. (1986). Aspects of evolutionary ecology of the Proteaceae with emphasis on the genus Leucadendron and its phylogeny. Unpublished PhD thesis.

Moll, E.J., Campbell, B.M., Cowling, R.M., Bossi, L., Jarman, M.L. & Boucher, C. (1984). A description of the vegetation categories in and adjacent to the fynbos biome. South African National Scientific Progress Report No. 83. CSIR, Pretoria.

Pate, J.S., Rasins, E., Rullo, J. & Kuo, J. (1985). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. Annals of Botany, 57, 747 - 750.

Rebelo, A.G. & Rourke, J.P. (1986). Seed germination and seed set in southern African Proteaceae: ecological determinants and horticultural problems. Acta Horticulturae, 185, 75-88.

Root, R.B. (1973). Organisation of a plant-arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs 43, 95-120.

Samson, D.A. & Werk, K.S. (1986). Size dependent effects in the analysis of reproductive effort in plants. The American Naturalist, 127, 667 - 680.

Silvertown, J & Rabinowitz, D. (1985). Reproductive decisions in the botanical world. New Scientist, 28 November, 32 - 34.

Sutherland, S. 1986. Floral sex ratios, fruit set and resource allocation in plants. Ecology, 67, 991 - 1001.

Taylor, H.C. (1978). Capensis. In M.J.A Werger, Biogeography and Ecology in Southern Africa, 1, 171-229. THe Hague.

Thwaites, R.N. & Cowling, R.M. (1988). Landscape - vegetation relationships on the Agulhas Plain, South Africa. Catena, 15, 333 - 345.

van Staden ,J. & Brown, N.A.C. (1977). Studies on the germination of South African Proteaceae - a review. Journal of South African Botany, 32, 291 - 298.

Wanntorp, H., Brooks, D.R., Nilsson, T., Nylin, S., Ronquist, F., Stearns, S. & Wedell, N. (1990). Phylogenetic aproaches in ecology. Oikos, 57, 119-132.

Witkowski, E.T.F. & Mitchell, D.T. (1987). Variations in soil phosphorus in the fynbos biome, South Africa. Journal of Ecology, 75, 1159 - 1171.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall Inc.

Section 7

7.0 APPLIED ASPECTS: THE IMPACT OF FLOWER AND CONE HARVESTING ON SEED BANKS AND SEED SET

7.1 ABSTRACT

Inflorescences and cones of serotinous (canopy-stored seed) Proteaceae are extensively harvested from natural stands in fynbos of the Cape Province, South Africa. This study investigates the impacts of harvesting on seed bank size and seed set of two Protea spp. and two Leucadendron spp. In all cases harvesting was found to deplete seed banks by up to 50%, and to reduce the following season's cone production. Remaining current year cones of the harvested Protea spp. had increased insect predation levels and unaltered or lower seed set, relative to unharvested plants. The latter is not consistent with the hypothesis that seed numbers are nutrient limited, since inflorescence harvesting represents a sink removal, and increased nutrients remaining in the plant would be available for increased seed set. Harvesting levels of not more than fifty percent of current inflorescences or cones, in alternate years, are recommended.

7.2 INTRODUCTION

Cape fynbos, which is a fire-prone shrubland confined to nutrient poor soils (Kruger 1979), is often dominated by shrubs relying on canopy-stored seed reserves (serotiny) for post-fire regeneration (Bond 1985). Fynbos communities on the Agulhas Plain (Thwaites & Cowling 1988) include many species which are economically valuable as cut flowers. Vast quantities of inflorescences and infructescences (cones) of many serotinous Proteaceae species are picked by an expanding wildflower industry. Since seed is a _sine qua non_ for post-fire population replacement of plants that are killed by fire, an understanding of harvesting impacts on seed banks and seed biology is needed to predict the effect of this harvesting on post-fire regeneration (Cowling, Lamont & Pierce 1986, van Wilgen & Lamb 1985).

Flower harvesting not only removes seeds, but also the nutrients they contain. Since both fynbos and south-western Australian Proteaceae grow on nutrient poor soils, and produce seeds rich in nutrients (Pate _et al_. 1985), it has been suggested that seed numbers are limited by nutrient availability (Lamont, Collins & Cowling 1985, Cowling, Lamont & Pierce 1986, Stock _et al_. 1989). Stock _et al_. (1989) found that inflorescence removal reduced subsequent inflorescence abortion in _Banksia laricina_ (Proteaceae), and attributed this to increased within plant nutrient availability. _Leucospermum parile_ (Proteaceae) takes up nutrients in winter and stores them in tap roots and above ground plant parts. Phosphorus and nitrogen are later translocated from these reserves and concentrated in the maturing seeds (Jongens - Roberts & Mitchell 1986). Esler et al.(1989) also found that the increase in phosphorus and nitrogen in maturing seeds came from extra - floral nutrient stores. It can therefore be inferred that harvesting of Proteaceae inflorescences prior to seed maturation leads to a relative increase in nutrients in the extra-floral plant reserves. If seed numbers are nutrient limited, then the remaining inflorescences would have increased seed numbers.

This study investigates the effect of harvesting on aspects of the seed ecology of proteaceous shrubs with a view to improving sustainable harvesting practices. Controlled harvesting was performed on previously unharvested populations of four serotinous species: Protea susannae Phill., P.obtusifolia Beuk ex Meisn., Leucadendron coniferum (L.) Meisn. and L.meridianum I.Williams. All are widely used by the wildflower industry. The effect of inflorescence or cone-harvesting on vegetative regrowth and on the following year's cone production was investigated for all species. Harvesting effects on seed set and seed predation levels was investigated for the Protea spp. only. The hypothesis that harvesting of the two Protea spp. at the immature inflorescence stage would result in increased seed set in the remaining infructescences due to increased within plant nutrient availability, was tested. The relationship between total cone number and canopy volume was compared between harvested and unharvested populations of all species except L.meridianum. Harvesting impacts on seed bank size were determined, and guidelines for harvesting management are discussed.

7.3 MATERIALS AND METHODS

7.3.1 Study area and study species

The study site is situated on the coastal lowlands of the Agulhas Plain ($34^{\circ}\ 35'$ S, $19^{\circ}\ 55'$ E), South Africa. Sampling was carried out in 18 year-old Proteoid Fynbos (Cowling et al. 1988) growing on soils derived from the Mio - Pliocene limestone of the Bredasdorp Formation (Thwaites & Cowling 1988). The climate of the area is mediterranean with 65% of the mean annual rainfall (452 mm) occurring between April and September.

The study species are seed-regenerating proteoid shrubs 1.5 to 3.0 m tall. Protea obtusifolia and Leucadendron meridianum co-occur on shallow, alkaline sands overlying limestone, and P.susannae and L.coniferum co-occur on the adjacent limestone-derived, deep, moderately acid, colluvial sands. The Protea spp. are monoecious, and inflorescences are picked between June and September, prior to seed maturation (Table 7.1). Female plants of the dioecious Leucadendron spp. are harvested in early autumn when inflorescences have matured into infructescences (cones). Table 7.1 also shows the seasons of flowering, seed maturation times, and the proportion of the total seed bank occurring in cones of different ages.

Table 7.1. Harvesting seasons and selected reproductive attributes of Agulhas Plain Proteaceae shrubs.

	Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Harvesting season	June-Sept	July-Sept	March	March
Flowering season	April-August	July-September	late August	early August
Seed maturation time (months)#	5	4	3.5	3.5
Season when current seed is mature	Sept-Jan	Nov-Jan	mid-Dec	mid-Nov
Contribution to seed bank (%)+				
Year 1	22	51	40	38
Year 2	34	28	35	25
> Yr 2	44	21	25	37

#Seed collected at intervals after flowering were considered mature when 90% of a test sample (75 seeds) germinated at 10°C: dark: 10hrs/20°C: light: 14hrs (unpublished data)

+Annual viable seed contribution as a percent of total seed bank (see Table 6.6)

7.3.2 The effect of experimental harvesting on cone production.

Thirty plants of each species were randomly chosen, labelled and experimentally harvested in ways similar to those practised by the wildflower industry:

i) *Protea* spp.

During the 1987 flowering season the number of current inflorescences was counted on all chosen individuals. Fifteen plants were left as controls, and fifteen were experimentally stem-harvested (cut 15-20 cm below the inflorescence) such that approximately 70% of the inflorescences were removed. The number of inflorescences produced in the flowering season after harvesting (1988) was counted.

ii). *Leucadendron* spp.

During March 1987 the number of current year cones was counted on all the chosen individuals. Each species was then divided into three groups of ten plants each - one left as a control, one for cone-harvesting (70% of current mature cones harvested immediately below the cones), and one for stem - harvesting (70% of current mature cones removed by cutting off the stem 15 - 20 cm below the cones). *Leucadendron* harvesting was done in March, 1987. The number of cones produced in the season after harvesting was determined in March 1988.

7.3.2 Size-dependent reproduction in harvested and unharvested populations.

In the unharvested populations all the cones on thirty plants per species were counted. Height and canopy diameter were measured, and canopy volumes were calculated as the volume of an ellipsoid ($4/3.pi.r_1.(r_2)^2$), where r_1=height/2; r_2=diameter/2). In similar aged vegetation in a neighbouring area where plants had been commercially harvested over the past five years, measurements of total cones and canopy volume were obtained for Protea susannae, P.obtusifolia and Leucadendron coniferum. Harvested L. meridianum plants were unavailable since this species had not been harvested for the past four years.

7.3.3 Effect of experimental harvesting on seed set and seed predation levels in Protea spp.

For each species five current cones per plant (15 harvested and 15 unharvested plants) were harvested in April 1988. The number of apparently viable (embryo-filled and plump), aborted, and predated seeds were counted. The viable and predated seed categories were each calculated as a percentage of the total (viable + aborted + predated) seed number.

7.3.4 Harvesting impacts on seed banks.

Annual contributions of viable seed to the seed bank of previously unharvested plants (Table 7.1) were used to calculate harvesting impacts on seed banks. Reduction in cone number due to harvesting , as well as the decrease in cone number in the season after harvesting, were incorporated in calculations of seed bank sizes in the year after harvesting. These were expressed as a percentage of the unharvested seed bank .

7.3.5 Statistical analyses.

In order to determine the effect of flower and cone harvesting on future cone production, the annual individual plant variation was accounted for by using the difference (change) for each plant between cone numbers of the pre- and post-harvest seasons. Student's t-tests were performed on these changes to investigate the significance of differences between stem-harvested and control plants. Mann Whitney tests were used to assess differences between proportions of viable and predated seed in unharvested and harvested plants.

Least squares regression analysis was used to determine the relationship between canopy volume and cone number in the harvested and unharvested populations. For all species canopy volumes in the two populations overlapped extensively. Differences in the slopes and elevations of regression for different population were determined using a t-test (Zar 1984).

7.4 RESULTS.

7.4.1 The effect of experimental harvesting on cone production.

There was no vegetative regrowth below the cut stems of any of the four species. In both *Leucadendron coniferum* and *L.meridianum*, cone-harvesting did not result in decreased cone production the following year (Table 7.2). Stem-harvesting, however, resulted in a significant decrease in cone numbers. Harvested *Protea obtusifolia* plants produced significantly less cones the season after picking. The decrease in cone production of *P.susannae* was greater in harvested plants, but this difference was not significant.

Table 7.2. The effect of inflorescence harvesting of *Protea susannae* and *P.obtusifolia*, and cone harvesting of *Leucadendron coniferum* and *L.meridianum*, on cone production the following year. Cone numbers of the current year's crop were determined immediately before (March 1987) and the year after (March 1988) experimental harvesting. Cone numbers of unharvested controls were determined at the same time. Plant populations had previously never been harvested. Data are means (SE). *n* = 15 plants for *Protea* spp.; *n* = 10 for *Leucadendron* spp. Significance of differences in cone numbers between controls and stem-harvested plants in the year after harvesting was determined by *t*-tests on the change in cone number between 1987 and 1988.

	Cone numbers		Change$
	1987 season	1988 season	
Protea susannae			
Unharvested	54.7 (12.2)	36.8 (7.1)	-17.9 (32.6)
Stem-harvested#	65.1 (12.4)	32.5 (6.6)	-32.5 (35.8)N.S.
Protea obtusifolia			
Unharvested	8.1 (2.0)	4.7 (1.5)	-3.4 (4.8)
Stem-harvested#	14.5 (2.6)	1.3 (0.6)	-13.2 (10.0)*
Leucadendron coniferum			
Unharvested	198.9 (34.0)	245.8 (72.8)	46.9 (171.8)
Cone-harvested+	238.0 (78.8)	271.0 (75.1)	33.0 (65.2)
Stem-harvested#	223.1 (46.2)	120.8 (36.2)	-102.3 (145.8)*
Leucadendron meridianum			
Unharvested	70.7 (13.7)	116.9 (19.4)	46.2 (38.7)
Cone-harvested+	99.9 (28.4)	136.9 (37.3)	37.0 (54.2)
Stem-harvested#	68.6 (12.1)	56.8 (21.9)	-11.8 (49.7)*

#Stems cut 15cm below inflorescences such that seventy percent of inflorescences removed

+Seventy percent of current cones harvested

$(1988 Cone numbers) - (1987 Cone numbers)

N.S.,Not significant. *P<0.05

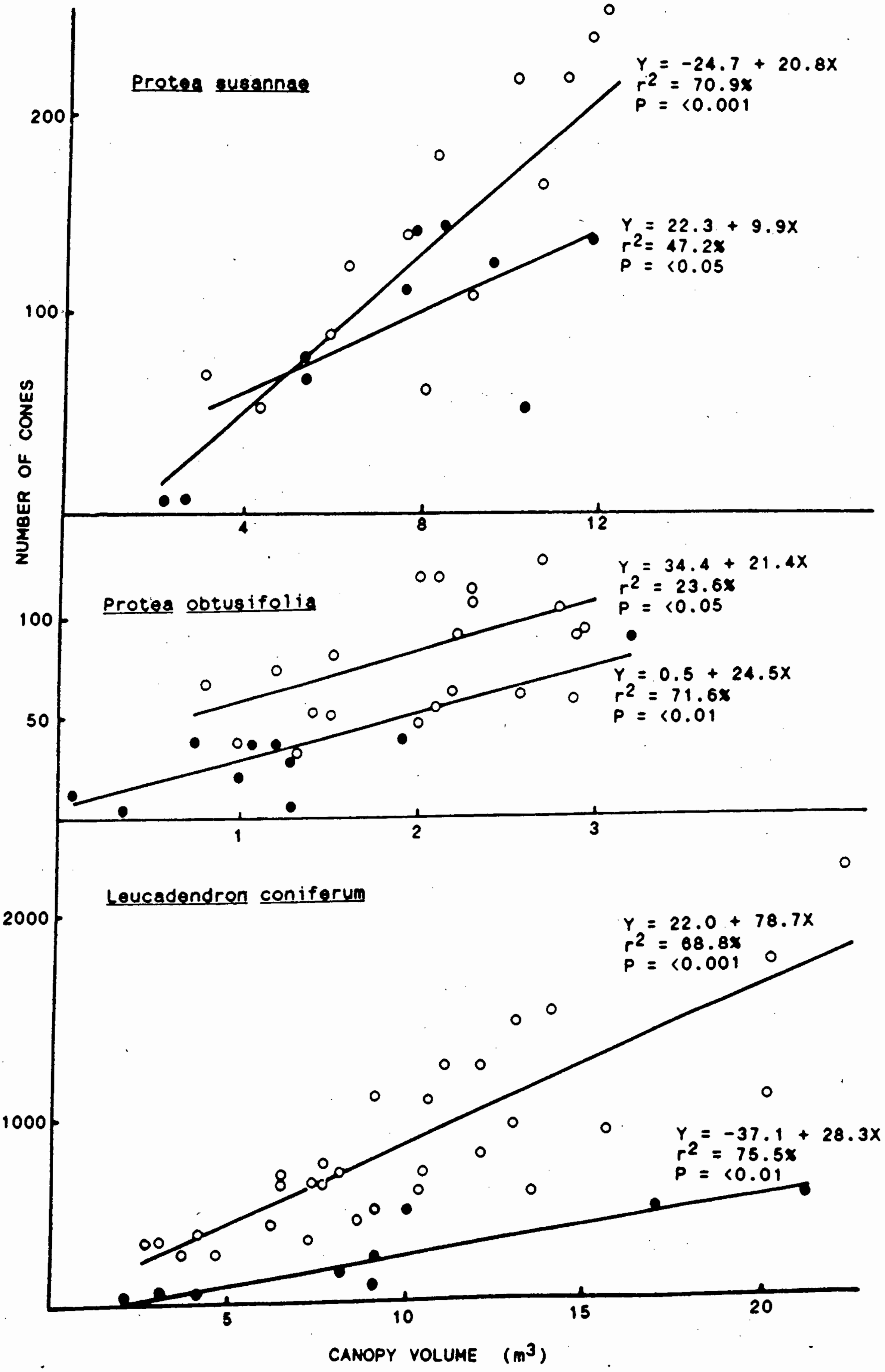


Figure 7.1. Relationships between total cone numbers per plant, and canopy volumes (m^3) in harvested and unharvested populations of *Protea susannae*, *P.obtusifolia* and *Leucadendron coniferum*. Harvested plants had been stem-harvested over the past 5 years.
● = harvested plants, ○ = unharvested plants

7.4.2 Size-dependent reproduction in harvested and unharvested populations.

Harvested shrubs had fewer cones than equivalent sized unharvested shrubs (Figure 7.1). The slopes of the regressions of harvested and unharvested were plants were significantly different for Leucadendron coniferum ($P<0.001$), but not for Protea susannae and P.obtusifolia. The elevations of regression for harvested and unharvested plants of the Protea spp. were significantly different ($P<0.05$ and $P<0.001$ respectively). The coefficients of determination, r^2, for harvested P.susannae and unharvested P.obtusifoloa were both low (less than 50%).

7.4.3 Effect of experimental harvesting on seed set and seed predation levels in Protea spp.

Whereas there was no difference in percent viable seed per cone (seed set) between harvested and unharvested plants of Protea obtusifolia, harvested P.susannae had a slightly lower (though statistically significant) proportion of seeds per cone than those of unharvested plants (Table 7.3). In both species there was an increase in predated seed in the cones of harvested individuals, although this change was not significant in P.susannae. The viable seed component of this increased predation in harvested plants (mean increase of 10.3% for P.obtusifolia and 14.3% for P.susannae) would be approximately 1.7% and 1.5% per cone respectively, assuming viable seed is indiscriminately eaten in proportion to mean percentages of viable seed present (unpublished data). Total viable seed of the

Table 7.3. Percent a) viable seeds and b) predated seeds per current cone of unharvested and remaining current cones of experimentally harvested plants of Protea susannae and P.obtusifolia. Plants were harvested such that seventy percent of current inflorescences were removed during their respective flowering seasons. Mature cones of the following season were analysed for viable, non-viable and predated seed components. Data are means (SE). Significance of differences between unharvested and harvested values was determined by Mann-Whitney tests.

	Protea susannae	Protea obtusifolia
a) Percent viable seeds per cone		
Unharvested	13.3 (2.1) (n = 15)	13.4 (3.1) (n = 13)
Harvested	11.1 (2.6)* (n = 14)	11.7 (2.6)N.S. (n = 14)
b) Percent predated seeds per cone		
Unharvested	13.5 (2.2) (n = 15)	23.3 (3.5) (n = 13)
Harvested	27.8 (3.5)* (n = 14)	33.6 (5.0)N.S. (n = 14)

n = number of plants (up to 5 cones per plant).

N.S., not significant. *P<0.05

Table 7.4. Proportion of unharvested seed bank remaining in the year after experimental harvesting. Post-harvest seed bank sizes are expressed as a percentage of unharvested seed bank sizes, and are mean values. n = 30.

	Protea susannae	Protea obtusifolia	Leucadendron coniferum	Leucadendron meridianum
70% current cones removed	75	57	65	62
70% current & 50% year 1 cones removed	61	44	53	54

harvested plants would then rise to 13.4% and 12.6% respectively.

7.4.4 Harvesting impacts on seed banks.

The most strongly serotinous species (i.e. those species which have the lowest contribution of current year's crop to the total seed bank) (Table 7.1) have seed banks which were least impacted by 70% harvesting of current inflorescences and cones (Table 7.4). For example, Protea susannae, relying on its current seed crop for only 22% of its seed stores, had its seed bank reduced to 75%, whereas P.obtusifolia with 50% in current cones, was reduced to 57%. Additional removal of fifty percent one year-old cones reduces seed banks of all species to about half, or less, their original value.

7.5 DISCUSSION

These results show that not only does harvesting reduce the standing crop of canopy-stored seed, but also that this reduction can be carried over into the following season due to the subsequent lowered cone production that occurs after stem-harvesting. In all species inflorescences are terminally produced and there was no vegetative regrowth below the cut stems. Therefore, it can be assumed that the post-harvest cone reduction is a consequence of the removal of vegetative modules containing future growth points, resulting in reduced canopy volume. Rebelo & Holmes (1988) surveyed unexploited and commercially-exploited populations of a serotinous fynbos shrub, Brunia albiflora, and found that harvested plants bore fewer infructescences than unharvested plants of the same size. This study also found fewer cones on plants in commercially harvested populations of Protea susannae, P.obtusifolia and Leucadendron coniferum than those on similar sized unharvested plants (Figure 7.1). This is also likely to be due to the repeated annual removal of terminal growth points. Canopy volume is reduced by repeated stem-harvesting (pers. observation), and these smaller sized plants have fewer cones than similar-sized unharvested plants. Whereas harvested plants of the Protea spp. had a constant number of cones less than similar-sized, unharvested plants (similar slopes), the difference increased significantly with increasing canopy volume in L.coniferum. This could be related to high picking intensities of the larger, prolific cone-bearing plants of the latter species. It is also possible that older cones fall off the plant (harvesting of current cones would lead to a greater proportion of the total cone number comprising older cones). Whatever the cause, the vulnerability of this species to stem-harvesting is heightened by this phenomenon. This relationship of plant size to reproductive output could be used as a guideline for recommended stem- harvesting intensities. Plants could be pruned to a certain minimum size pre-chosen as that bearing an adequate number of cones (i.e. containing sufficient seed for post-fire regeneration). Samson & Werk (1986) stress this approach of noting size-dependent effects on reproduction.

If viable seed number is determined by available nutrients, it would be expected that seed set per cone in remaining cones of harvested Protea spp. would be higher than those of unharvested control plants. Experimentally harvested plants had seed set per cone either similar to (P.obtusifolia), or lower than (P.susannae) those of unharvested plants (in both cases effectively resulting in lower current seed numbers per harvested plant, since cone numbers are less). The estimated viable seed component of increased seed predation found in harvested plants would not change this result. Inflorescence harvesting represents a nutrient sink removal, and this did not result in an increase the number of viable seed forming in the fewer remaining inflorescences. This is not consistent with the hypothesis that seed numbers are determined by nutrient levels in these two species. It is, however, possible that in evolutionary time, low soil nutrients have fixed a limit to seed number. These conclusions differ from those of Stock _et al_ (1989) who found that experimental removal of inflorescences in _Banksia laricina_ led to decreased abortion levels of the remaining blooms, resulting in unaltered seed numbers _per plant_, compared to unharvested controls. Infloresence abortion in our two _Protea_ spp. is minimal, so mechanisms for altering seed numbers per plant (if, in fact, they do exist) would be different, with possible change being affected at the level of number of seed per cone rather than in numbers of infructescences per plant. Ayre & Whelan (1989) have suggested that there are independently operating mechanisms for controlling fruit set via excess flowers per inflorescence and excess inflorescences per plant . Alternative explanations for the low seed set in Proteaceae include pollen or pollinator limitation, lack of space, high insect predation levels, or post-zygotic abortion to produce high quality seeds in an outcrossing system (Bierzychudek 1981, Collins & Rebelo 1987, Zimmerman & Pyke 1988, Ayre & Whelan 1989, Wallace & O'Dowd 1989). Low seed set in _Protea_ spp. has also been suggested to be an anti-predation strategy (Coetzee & Giliomee 1987).

Protea obtusifolia seed predation levels were higher in the remaining cones of harvested plants, than in those of unharvested controls. The same trend (though not significant) was found for _P.susannae_. It is possible that insect predators of low mobility emerging from older cones on the same plants would concentrate on the lower numbers of cones. This finding is in contrast to the decreased predation that has been reported to occur with successive annual harvesting of populations of _Protea repens_ over five years, when the number of predated blooms decreased from eighty to fifteen percent (A.Gray, pers. comm.). This finding can be attributed to the removal of sources of insect infestation in that there are fewer old cones. This phenomenon has been also been reported in harvested _P.magnfica_ (= _barbigera_) plants (Myburgh, Starke & Rust 1974).

In serotinous species, such as those in this study, the proportion of seed banks removed by flower or current cone harvesting depends on the extent of older stored seed reserves (ie the degree of serotiny). It has been shown that canopy-stored seed remains viable for at least four to five years in cones (Section 3, Table3.2). The size of these reserves depend in turn on the intensity and frequency of harvesting in earlier years. In this study, with controlled harvesting on previously unharvested plants of known patterns of annual seed storage , seed loss has been calculated. Since harvesting by the wild flower industry is repeated in successive years, thus altering these patterns of seed storage, it is more difficult to make generalisations about the extent of seed bank depletion. However, it was shown that seed bank reductions can be severe in weakly serotinous species, with seed banks being decreased by about forty percent when harvested for the first time (seventy percent of current cones removed). Additional removal of older cones (fifty percent of one year-old cones) further reduced their seed banks to half, or less, their original size. Repeated annual harvesting could thus result in serious seed bank depletion. Protea susannae could be more resilient to harvesting since it relies on current seed production to a lesser extent (twenty two percent of seed bank) than the other species (thirty eight to fifty one percent). In addition, this species is not in high demand, unlike the co-occurring Leucadendron coniferum. The latter species retains only twenty five percent of its seed bank in cones older than 2 years, and the possibility of a severe harvesting-induced reduction in seed bank exists. According to lottery model theories seeds randomly occupy post-fire space, and co-existence is mediated by reproductive similarity, such as similar seed numbers (Fagerström 1988). In harvested populations such a post-fire lottery would favour P.susannae. Similarly, sustained harvesting of P.obtusifolia would reduce its ability to compete for establishment sites with the unharvested L.meridianum. The increasing numerical advantage of P.susannae and L.meridianum seedlings over several post-fire regeneration events would lead to the eventual elimination of L.coniferum and P.obtusifolia respectively, in harvested areas. There is evidence of a decline in post-fire recruitment of L.coniferum in mixed stands with P.susannae (R.I.Yeaton & R.M.Cowling, unpublished data)

It is not known how many seeds are required for adequate post-fire regeneration. There is much variability in Proteaceae post-fire seedling : pre-fire parent ratios. This variability is largely unexplained (Midgley 1989), though some has been attributed to season of burn (Bond, Vlok & Viviers 1984, van Wilgen & Viviers, 1985). The species in this study can have several thousand canopy-stored seed per plant, yet only one successful seedling is needed for plant replacement. It is possible that seed not lost through harvesting would be lost in density dependent seedling interactions. At the other extreme, poor establishment under adverse conditions could lead to local extinction. It is suggested that seed bank sizes should not be decreased by more than fifty percent. This approximates the maximum degree (22% to 51%, for these species) by which unharvested seed banks would be reduced in the event of an unseasonable fire in late spring/early summer, before the current seed has matured (Table 7.1). Harvesting levels of not more than fifty percent of current inflorescences or cones are recommended. In light of the subsequent lowered cone production in the post-harvest year, it is further suggested that stem-harvesting be performed in alternate years. This would allow some measure of vegetative and subsequent reproductive restoration.

7.6 REFERENCES

Ayre, D.A. & Whelan, R.J. (1989). Factors controlling fruit set in hermaphroditic plants: studies with the Australian Proteaceae. Trends in Ecology and Evolution 4, 267-272.

Bierzychudek, P. (1981). Pollinator limitation of plant reproductive effort. American Naturalist, 117, 838-840.

Bond, W.J., Vlok, J. & Viviers,M. (1984). Variation in seedling recruitment of Cape Proteaceae after fire. Journal of Ecology 72, 209 - 221.

Bond, W.J. (1985). Canopy stored seed reserves (serotiny) in Cape Proteaceae. South African Journal of Botany, 51, 181-186.

Coetzee, J.H. & Giliomee, J.H. (1987). Seed predation and survival in the infructescences of Protea repens (Proteaceae). South African Journal of Botany 53, 61-64.

Collins,B.G. & Rebelo,T. (1987). Pollination Biology of the Proteaceae in Australia and southern Africa. Australian Journal of Ecology, 12, 387-422.

Cowling, R.M., Lamont, B.B. & Pierce, S.M. (1986). Seed bank dynamics of four co-occurring Banksia species. Journal of Ecology, 75, 289 - 302.

Cowling, R.M., Campbell, B.M., Mustart, P.J., McDonald, D.J., Jarman, M.L. & Moll, E,J. (1988). Vegetation classification in a floristically complex area: the Agulhas Plain. Journal of South African Botany, 54, 290-300.

Esler, K.J., Cowling R.M., Witkowski E.T.F. & Mustart P.J. (1989). Reproductive traits and accumulation of nitrogen and phosphorus during the development of fruits of Protea compacta R.Br. (Calcifuge) and Protea obtusifolia Beuk. ex Meisn. (Calcicole). New Phytologist, 112, 109 - 115.

Fagerström, T. (1988). Lotteries in communities of sessile organisms. Trends in Ecology and Evolution, 3, 303-306.

Jongens - Roberts, S.M. & Mitchell, D.T. (1986). The distribution of dry mass in an evergreen fynbos shrub species Leucadendron parile (Salisbury ex J. Knight) Sweet (Proteaceae), at different stages of development. New Phytologist, 103, 669-683.

Kruger,F.J. (1979). South African heathlands. In: Specht, R.L.(ed), Ecosystems of the world. Vol 9A, Heathlands and related shrublands: Descriptive studies. Elsevier, Amsterdam, 19-80.

Lamont, B.B., Collins,B.G. & Cowling, R.M. (1985). Reproductive biology of the Proteaceae in Australia and South Africa.

Proceedings of the Ecological Society of South Africa 14, 213-224.

Midgley, J.J. (1989). Season of burn of serotinous Proteaceae: A critical review and further data. South African Journal of Botany 55, 165 - 170.

Myburgh, A.C., Starke,L.C. & Rust,D.J. (1974). Destructive insects in the seed heads of *Protea barbigera*. Journal of the Entomological Society of South Africa, 37, 23-29.

Pate, J.S., Rasins, E., Rullo, J. & Kuo, J. (1985). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. Annals of Botany, 57, 747 - 750.

Rebelo, A.G & Holmes, P.M. (1988). Commercial exploitation of *Brunia albiflora* (Bruniaceae) in South Africa. Biological Conservation, 45, 195-207.

Samson, D.A. & Werk, K.S. (1986). Size dependent effects in the analysis of reproductive effort in plants. American Naturalist 127, 667 - 680.

Stock, W.D., Pate, J.S., Kuo,J & Hansen, A.P. (1989). Resource control of seed set in *Banksia laricina* C.Gardner (Proteaceae). Functional Ecology 3, 453 - 460.

Thwaites, R.N. & Cowling, R.M. (1988). Landscape - vegetation relationships on the Agulhas Plain, South Africa. Catena 15, 333 - 345.

van Wilgen, B.M. & Lamb, A.J. (1985). The wildflower picking industry in relation to mountain catchment in the fynbos. Acta Horticulturae 185, 181 - 187.

van Wilgen, B.W. & Viviers, M. (1985). The effects of season of fire on serotinous Proteaceae and the implications for fynbos management. South African Forestry Journal 133, 49 - 53.

Wallace, D.D. & O'Dowd, D.J. (1989). The effects of nutrients and inflorescence damage by insects on fruit set by Banksia spinulosa. Oecologia 79, 482-488.

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall Inc.

Zimmerman, M. & Pyke, G.H. (1988). Reproduction in Polemonium: assessing the factors limiting seed set. American Naturalist 131, 723-738.

8.0 GENERAL DISCUSSION

8.1 SOIL AND PHYLOGENETIC EFFECTS

The underlying theme of investigating the effect of soil regime in each of the two species-pairs, without the constraints of phylogeny, revealed a dichotomy between the young (seed and seedling) and the mature plant stages.

8.1.1 Soil regime, seeds and seedlings

Studies attempting to determine the ecological basis for seed size optimisation have shown that there is much inter- and intra- specific variation (Silvertown 1989). A large study investigating the ecological and taxonomic correlates of seed mass variation in 648 Indiana dune species showed that the taxonomic component accounted for as much of the variation in seed mass as did ecological factors (Mazer 1989). This study also showed that at a geographic scale there was no relation between seed size and any habitat-specific ecological attribute. Lee & Fenner (1989) excluded taxonomic constraints by looking at twelve closely related grass species over a geographic range of soil fertilities in New Zealand, and found a negative correlation between soil fertility and seed size. This study has excluded both taxonomic constraints and geographic range variability by looking at two pairs of sister taxa on adjacent, different habitats. Furthermore, instead of using broad-scale descriptions of soil fertility and moisture, the soil surface nutrient and moisture regimes are clearly described at the scale of seed size. I showed a relationship between soil regime and seed size. Larger seeds within both genera on the colluvial sands were interpreted as selection for successful establishment of the subsequent larger seedlings in this soil with its lower nutrient and water concentrations than the limestone (Section 5). A putative phylogenetic constraint imposing smaller seed size in the Leucadendron spp. is suggested to be countered by their increased root (relative to shoot) lengths than the larger-seed Protea spp.

A relationship between soil regime and seedling establishment was also found, and was interpreted as being important in determining adult distribution patterns (Section 2). It was shown that only the larger-seeded species grew into seedlings able to survive on the drier colluvial sands, in contrast with the high mortalities of the smaller-seeded limestone endemics on these soils.

Species diversity patterns indicate that the turnover between habitats is a major factor in determining landscape-level species richness in South African fynbos (Kruger & Taylor 1979, Linder 1985, Cowling 1990), Australian kwongan (Bond & Goldblatt 1984, Lamont, Hopkins & Hnatiuk 1984) and tropical rain forests (Gentry 1988). This study actually investigated one of the processes determining beta diversity. Differences in soil habitat (nutrients and/or moisture), leading to selection for different seed sizes within taxonomic groups, would be starting points for subsequent speciation.

The relation between soil regime and seed germination showed a different pattern in that there was higher (Table 2.2) and less variable (Table 4.1) seedling emergence of all four study species on the limestone soil than on the colluvial sands. This was attributed to the higher moisture contents of the former soil than the latter. In hindsight this is not surprising since water is the major determinant in germination (Mayer & Poljakoff-Mayber 1975).

On the drier colluvial sands there were differences in emergence patterns between genera with the _Protea_ spp. having less variable emergence patterns than the _Leucadendron_ spp. (Table 4.1). The low water-binding properties of the colluvial sands are interpreted as leading to high micro-habitat variability in soil-surface water contents. Laboratory experiments showed that whereas seeds planted in each soil imbibed equal amounts of water, on drying the seeds in the limestone soil lost less water than those in the colluvial sand (see 3.4). Thus the colluvial sands appear to buffer seed-imbibed water loss to the atmosphere less efficiently than the limestone soil. Other studies have shown an experimental relationship between micro-habitat variability and seed germination (Harper, Williams & Sagar 1965, Hamrick & Lee 1987, Fowler 1988). This study has shown how soil properties _per se_ can lead to differences in seed-imbibed water levels, that in turn would influence germination. The more substantial pericarp structure in relation to embryo size of the _Protea_ spp. than the _Leucadendron_ spp. is suggested to account for the increased and less variable emergence patterns of the former on the colluvial sands. Of interest in this regard are preliminary findings which showed that after five days imbibition the pericarp of _Protea susannae_ increased its dry weight by 94% compared to the embryo which increased by 32%. Comparable data for _P.obtusifolia_ were 44% and 43% respectively. The function of the pericarp in seed-water relations needs further investigation (see 8.5). Pericarp morphology of Protea spp. has been investigated with regard to dispersal properties only (Manders 1986, Bond 1988). Seed coat or pericarp structure in Proteaceae is usually cosidered in relation to dormancy (Brits 1986, van Staden & Brown 1977). I suggest that pericarp:embryo size relationships play a role in determining the seed imbibition and hence germination success. Mazer (1989) has suggested this to be generally important for small seeds which have high seed coat thickness relative to seed volume.

8.1.2 Soil regime and adult plants

Whereas the questions asked about the relationship between soil regime and seeds and seedlings were relatively well defined, questions were more tentatively framed with regard to soil regime and adult traits. It was not known whether the larger volume of the colluvial sands would offset its lower nutrient concentrations, making it potentially more resource-rich than the higher nutrient concentrations of the limestone soil which occurs in pockets of small volume. In addition, the water regime at the rooting depths of the adults is not known. Thus, in a sense the plants growing on these soils were used as phytometers. The question was asked whether the species reflected any difference in their reproductive traits that could be related to the differences in soil regime. It was found on the colluvial sands that in both species-pairs the plants occurred at lower densities, were larger, and had greater numbers of cones and seed per plant, than species on the limestone (Section 6). Apart from these size-related properties, there were no consistent differences in reproductive traits. It was concluded that the different soil regimes had no influence on the investigated reproductive traits. However, soil regime was shown to be related to seed size (Section 5), but this relationship did not extend to other reproductive traits investigated.

Another way of looking at the relationship between soil regime and reproductive traits would be to determine whether trade-offs between vegetative and reproductive components (sensu Silvertown 1987) were different on the two soil types. If soil nutrients on either habitat were limiting, then the nutritionally expensive reproductive processes would result in a larger trade-off of decreased vegetation on the more resource-limiting soil. Assuming that the nitrogen- and phosphorus-rich embryos (van Staden & Brown 1977, Pate et al. 1985, Jongens-Roberts & Mitchell 1986) represent the main reproductive cost, the gross mass of embryo produced per plant (i.e. including seed that were lost to predators) was calculated, and related to plant canopy volume (Table 8.1). Here, too, there was no consistent trend within species-pairs across soils. Protea susannae with potentially more available nutrients, showed decreased reproduction per unit of canopy volume relative to P.obtusifolia, indicating, perhaps, that the cost of exploring large soil volumes in order to obtain nutrients in the colluvial sands resulted in a trade-off in decreased reproduction.

Table 8.1. Relationship of embryo production to canopy volume of co-occurring *Protea susannae* and *Leucadendron coniferum*; and *P.obtusifolia* and *L.meridianum*.

	Protea susannae	*Leucadendron*[Ø] *coniferum*	*Protea obtusifolia*	*Leucadendron*[Ø] *meridianum*
Canopy volume (m^2)[#]	14.7	9.9	2.7	2.0
Cones/plant[#]	252.0	818	92	363.0
Seed/cone[$]	17.3	40.7	42.6	11.3
Seed/plant[+]	4360	33293	3919	3803
Embryo mass (mg)[*]	16.8	9.3	12.0	6.1
Embryo mass per plant (g)[@]	69.8	309.6	47.0	23.2
Embryo mass/ canopy volume (g/m^2)	4.7	31.3	17.4	11.6

[#] Means data (from Section 6.4)

[$] Mean seed number per cone in unpredated, current cones (from Section 6.7)

[*] Means data (from Section 5.2)

[+] (Cones/plant) x (seed/cone) i.e. gross seed production, excluding seed predation

[@] (Seed/plant) x embryo mass

[Ø] Female data

However, the reverse was shown in the Leucadendron spp., negating the strength of a soil regime-related explanation. The high reproductive output of L.coniferum is most probably related to demographic selection (See 8.2). It is predicted that this cost be reflected in high adult mortalities of this species.

The lack of increased seed set in remaining infructescences of the two Protea spp. after inflorescence sink removal (Section 7.4.3) is further indication of a lack of relationship between soil nutrient regime and reproduction. The low seed set of hermaphroditic Proteaceae in Australia and South Africa have been reviewed by Ayre & Whelan (1989) who have identified both proximate (ecological and physiological) and ultimate (past selection for high flower numbers) reasons for this. Proximate causes include resource nutrient limitation, and this would be particularly relevant for plants growing on the nutrient poor soils of Australia and South Africa (Lamont, Collins & Cowling 1985). Manipulative experiments on seed set in Australian Proteaceae have shown support for the nutrient limitation hypothesis (Stock et al. 1989), a different finding from this study. The lack of any direct relationship between soil nutrient status and reproductive trait (other than seed size) suggests that low soil nutrients might have been an ultimate cause of Proteaceae reproductive traits, but that proximate relationships are more likely to be determined by other factors (fire-related, or demographic).

8.2 THE RELEVANCE OF SEED AND SEEDLING BIOLOGY FOR UNDERSTANDING FYNBOS POPULATION STRUCTURE

These differences found in the relationship between seed and seedlings, and adult plants, to their soil environment, stress the importance of understanding the biology of the regeneration stages in order to fully understand adult population patterns (Grubb 1977). It has been suggested that knowledge of pre-burn seedbank sizes would be important for understanding and predicting post-fire recruitment patterns (Cowling, Lamont & Pierce 1986, Lamont & Barker 1988, Cowling 1989). An integrated approach has been preferred by others who examine the end result only. Post-fire seedling:parent ratios are determined in order to gauge the effect of season of burn (Bond, Vlok & Viviers 1984, van Wilgen & Viviers 1985, Midgley 1989) and these studies have shown that winter and spring burns generally lead to poor recruitment. Experimental evidence suggests that successful recruitment after summer and autumn burns is due to a shortened period between seed release and winter germination, and hence less seed predation, than would occur after late winter or spring burns (Bond 1984). This approach implies that post-burn factors are of more importance than pre-burn ones, and discounts Jordaan's (1949) suggestion that variation in pre-burn seed numbers due to season of seed maturation influences post-burn seedling numbers. However, a collated data set on fynbos seasonal regeneration responses shows that despite the above explanations there is still much unexplained variation in seedling:parent ratios (Midgley 1989).

In addition, Bond, Vlok & Viviers (1984), who found a decline in seedling:parent ratios with increasing pre-burn plant density, also found considerable fire to fire variation. This led them to suggest that the size of pre-burn seed reserves, as well as post-fire events, could be determinants of recruitment success.

It has been shown that when conditions are adverse for seedling establishment, a doubling of seed densities more than doubles seedling numbers (Harper & Chancellor 1959). In the light of this , knowledge of seed bank sizes, together with phenology of seed maturation, is needed in areas with high summer seedling mortality in order to interpret the effect of season of burn on post-fire regeneration. Summer mortality during the often dry, windy summers can be high in this lowland Agulhas area (40% mortality of two year-old seedlings on the colluvial sands after a dry summer, (unpublished data)). Maximum post-fire seed release, and hence seedling numbers would be needed to buffer these high losses. This high summer drought mortality contrasts with the low values (ca. 7%) reported for Proteaceae seedlings in mountain fynbos (Midgley 1988), precluding the need for large seed banks in the latter areas. Seed bank sizes of Proteaceae occurring in the mountain fynbos are not known.

Pre-burn seed bank sizes, and the associated reproductive strategies in achieving this, are not usually known. The results of this thesis show much variation amongst these four species, both in size of canopy-seed stores, as well as in strategies employed to reach them. The large individual plant

Table 8.2. Seed bank characteristics of co-occurring _Protea susannae_ and _Leucadendron coniferum_; and _P.obtusifolia_ and _L.meridianum_.

	Protea susannae	_Leucadendron coniferum_	_Protea obtusifolia_	_Leucadendron meridianum_
Seed per plant#	3178	24 024*	1602	2550*
Plant density (plants/100 m^2)#	8.5	4.5 2.0*	19.4	13.8 6.5*
Population seed numbers per hectare	2.7×10^6	4.8×10^6	3.7×10^6	1.5×10^6
Proportion of seed bank in current cones (%)#	21.8	40.0	50.8	38.4

#Means data, from Section 6.4
i.e. nett seed numbers, taking seed predation into account

*Female plant data

seed stores of Leucadendron coniferum (24 000 per female plant, or 10 677 per plant taking into account proportions of male and female plants of this dioecious species) compared to Protea susannae (3 178) were offset by their different plant densities to give population seed numbers (seeds per hectare) of 4.8×10^6 and 2.7×10^6 respectively (Table 8.2). Of these 40% (L. coniferum) and 21.8% (P. susannae) were contributed by year one cones. The lower plant density of L.coniferum than P.susannae, considering the 3.3-fold differences in seed numbers per plant, is in itself an indication of prior adverse recruitment of the former species, and could be explained as follows. The current seed crop of L.coniferum is mature by mid-December only, whereas P.susannae with its prolonged flowering season has a substantial proportion of its current seed crop mature by late September (Table 8.3). This, together with the higher degree of serotiny of the latter, would result in seed numbers of P.susannae being less affected by spring burns in late summer (Sept. to Nov.) than L.coniferum. However, the ability of a proportion of L.coniferum seed to germinate at 15/30°C, suggested to extend the germination period of this species into the warmer months more effectively than the other species (see Section 3.5), would in some measure offset the impact of this seed bank decrease.

In addition to such knowledge of seed banks and phenology of current seed maturation, other aspects of seed and seedling ecology provide useful insight. Field germination trials of planted seed showed that P.susannae had higher emergence than L.coniferum (Table 8.4). Furthermore, L.coniferum has lower seed mass, and consequently lower seedling root and shoot dry mass, than *P.susannae*. Field seedling mortality after two summers was higher in the former (51.4%) than in the latter

Table 8.3. Phenology of flowering and seed maturation of co-occurring *Protea susannae* and *Leucadendron coniferum*; and *P.obtusifolia* and *L.meridianum*.

	Protea susannae	*Leucadendron coniferum*	*Protea obtusifolia*	*Leucadendron meridianum*
Flowering season[#]	April to August	late-August	July to September	Early-August
Months for seed to mature[$]	5	3.5	4	3.5
Period when current seed matures[+]	September to January	Mid-December	November to January	Mid-November

[#] Monthly number of open inflorescences were calculated as a percent of the maximum recorded number (*n*=20 individuals). Period of flowering was taken when >10% such flowering occurred. Values for *Leucadendron* spp. represent female data.

[$] Seed were considered mature when >90% of a test sample (*n*=75 seeds) germinated at a 10/20°C alternating dark/light for 14/10 hours.

Table 8.4. Seed and seedling characteristics of co-occurring _Protea susannae_ and _Leucadendron coniferum_; and _P.obtusifolia_ and _L.meridianum_.

	Protea susannae	_Leucadendron coniferum_	_Protea obtusifolia_	_Leucadendron meridianum_
Seed mass$ (mg)	36.5	12.6	23.2	9.0
Root dry mass (mg)#	109.1	43.7	72.9	34.3
Shoot dry mass (mg)#	125.3	78.0	73.2	56.7
Field shoot dry mass (g)*	0.55	0.39	0.35	0.27
Seedling emergence (% of seed planted)*	59.4	46.3	48.8	45.6
Seedling mortality (% of emerged seedlings)*	36.5	51.4	10.3	12.4

$Means data, from Section 5.4

#Means data of laboratory grown seedlings, from Section 5.4

*Means data of field emergence of _P.susannae_ and _L.coniferum_ on colluvial sands, and _P.obtusifolia_ and _L.meridianum_ on limestone soil, taken from Section 2.4

(36.8%). Incorporating these results into a simple calculation shows that the potential 3.3-fold advantage of the prolific seed producer, L.coniferum, is reduced to only 1.3 times that of P.susannae. The large seed bank sizes of Leucadenron coniferum permit co-existence with its "superior" seedling congener Protea susannae. Since these species commonly co-exist, nett recruitment would be the same for both. Co-existence of species differing in regeneration niche attributes (Grubb 1977) have been shown by modelling to occur when the inferior species (in this case L.coniferum with lower seedling emergence and higher seedling mortality than P.susannae) can persist by being superior in seed production (Fagerström & Agren 1979).

Long-term co-existence of these species-pairs would probably be ensured by stochasticity in fire regime and post-fire climatic conditions (Chesson & Huntly 1989). However, in the short-term, population fluctuations could occur. For example, the occurrence of very dry summers in the first few years after a spring (September to November) burn might lead to poor recruitment of Leucadendron coniferum (current seed not mature (Table 8.3) and greater seedling susceptibility to summer drought (Table 8.4)), whereas Protea susannae (some current seed mature, and lower seedling drought mortality) would have better recruitment. At the other extreme, a cycle of less water-stressed summers after an autumn burn (March to April) would result in favourable recruitment of both species. According to lottery theory (Fagerström 1988) the random allocation of new individuals (in this case, seed) in (post-fire) space would favour the prolific seed producer L.coniferum. Thus, should the latter cycle be repeated several times, population densities of L.coniferum should increase to the point of excluding P.susannae. A modelling approach to compute such effects of fire regime and climate variations on post-fire recruitment, and on the co-existence of these species, would be useful.

The same arguments hold for co-occurring Protea obtusifolia and Leucadendron meridianum. However, compared to the P.susannae - L.coniferum pair, there is less of a discrepancy in seed numbers per plant (2550 per female, or 1200 per plant taking into account male and female plant proportions for L.meridianum; 1602 for P.obtusifolia) and degree of serotiny (Table 8.2), greater synchrony in seed maturation timing (Table 8.3), as well as more similar seedling emergence and mortality (Table 8.4). I thus predict that co-existence, as well as plant population densities, of these two species will be more stable, irrespective of season of burn or summer drought patterns. This would substantiate lottery theory which predicts that co-existing species should have similar reproductive output (Fagerström 1988).

I conclude that knowledge of pre-burn seed stores, together with aspects of post-burn seed and seedling ecology , can give predictive insight into post-fire recruitment and co-existence population patterns. These predictions could be tested using post-fire seedling:parent data, in relation to season of burn and post-fire weather data.

8.3 HARVESTING IMPACTS

Whilst the study quantified the impact that a selected level of harvesting would have on seed banks, I could provide no absolute answer as to what level, and frequency, of harvesting would be detrimental to post-fire regeneration of the species concerned. Since the answer to " how many seeds is enough" depends on other factors, such as fire season, influencing recruitment, the level of harvesting recommended would need follow-up asessment of post-fire recruitment (seedling:parent data).

An aspect that needs to be considered is the effect of harvesting on population genetic variability. Since the largest, least predated inflorescences are usually harvested, this could represent a selective removal of genes. As the post-fire environment would vary after different fire cycles and different weather patterns, selection pressures would also vary. Maximum offspring genotypic variability would be an advantage in these situations (Frost 1984), and the effects of selective gene removal could be noticed in the long-term only. Future research is needed to investigate this.

8.4 LIMITATIONS

In the investigation into regeneration niche requirements, I determined mid-summer shoot water potentials (Table 2.6), and leaf conductance and transpiration rates (Table 2.7) in order to test whether water stress was the cause of seedling mortality in the field reciprocal transplants on the two soil types. These were done during the second summer of establishment, and no differences were found between seedlings on either soil. In a sense this was akin to shutting the stable door after the horse had escaped, in that the unsuccessful (possibly water-stressed) seedlings would have been the ones that had died, thus precluding measurements. An attempt to determine the water status of seedlings during the first summer before most of the seedlings had died, was thwarted by heavy, unexpected rain, and no subsequent dry periods before the winter. A further limitation of this part of the study was the lack of root, as well as shoot, mineral determinations. Root excavations would have disturbed the remaining planted seedlings. Since roots are important in iron metabolism (Brown & Jolley 1989), nutritional deficiencies not picked up in shoots might have been found in root measurements. I stress that any eco-physiological investigations in this study were performed in order to understand ecological phenomena, and not in order to elucidate detailed physiological mechanisms.

In Section 4, I found differences in laboratory determined germination rates of different ages of canopy-stored seed. These were interpreted as a bet-hedging strategy which would ensure maximal field germination and emergence. Additional field emergence studies of these seed categories would have provided more definite evidence of the importance of this phenomenon. The investigation into adult plant reproductive strategies on different soil types was based on the fact that similar soils supporting the same Proteaceae spp. in the same and separate areas of the Agulhas Plain, showed definite differences in soil nutrient concentrations between limestone and colluvial sands

(Table 6.2). The former had significantly higher concentrations of carbon, nitrogen and total phosphorus than the latter. Determinations performed on soils of my study site showed less dramatic differences, and phosphorus concentrations were the same in both soil types (Table 6.1). Repeat determinations gave the same results. Phosphorus determinations on limestone soil sampled from the same study area a year earlier (Esler _et al_ 1988) gave results that were four times higher than my determinations. I have no explanation for these descrepancies, and continued with my interpretations on the assumption that the colluvial sands had lower concentrations of soil nutrients than the limestone soils.

8.5 FURTHER RESEARCH

The role of the pericarp in seed-water relations of Proteaceae spp. is an area that needs further investigation. The anatomy of different pericarp structures in relation to water-binding properties could provide an answer as to why there is so much variation in seed morphology in this widely distributed family.

Further investigation into aspects of seed germination and early seedling emergence ecology is required, especially for species growing at the arid fringe of the fynbos along soil and moisture gradients. This could provide answers to questions relating to species and population distribution patterns in these areas. If regeneration stages are sensitive to temperature, and temperature-related moisture conditions, then the predicted global warming and its associated climate changes (Tyson 1991) would result in highly specialist species being vulnerable to such climatic change. Detailed investigations into seed and seedling ecology of these species would result in predictions about vegetation changes under different climate scenarios (Bond & Richardson 1990).

The selective harvesting of one of the species in either of the co-occurring Protea - Leucadendron pairs was suggested to have implications for co-existence (Section 7). Lottery theory predicts co-existence as a result of reproductive similarity (Fagerström 1988), such as seed numbers. Excessive harvesting of one species of a co-occurring pair, combined with unfavourable fire season would led to selective elimination of the species concerned. A modelling approach to investigate these effects would be important in assessing such harvesting impacts on seed banks and continued co-existence of populations. The applicability of lottery modelling for fynbos species has been demonstrated (Laurie & Cowling, in preparation).

The role of competition in determining seedling co-existence has been mentioned in Section 2.5.2. I predicted that on both the limestone and colluvial sands, the ultimate outcome of species survival would be determined by competitive effects, and that species with lower relative growth rates would be eliminated. The importance of such competive effects needs clarifiation and is currently being investigated by M.B. Richards (University of Cape Town).

Throughout this study, trends within each of the two closely related species-pairs were correlated with soil regime differences. Further confirmation of trends such as seed size, for example, could be tested for other taxa (eg. Rhamnaceae, Rutaceae) which have sister taxa on both soil types.

During the (very) tedious task of sorting out and counting the fertile and infertile seed of the Protea spp., I noticed that the fertile seed appeared to occur in small clusters on the receptacle. Since insects feed non-discriminately on both fertile and infertile seed, low seed set in the Protea spp. has been suggested to be a means of reducing pre-dispersal predation of serotinous species (Coetzee & Giliomee 1987). I am currently testing the hypothesis that seed are clustered, and not randomly arranged, on receptacles of Protea spp. in order to provide an aggregated refuge for nutritionally expensive fertile seeds.

8.5 REFERENCES

Ayre, D.A. & Whelan, R.J. (1989). Factors controlling fruit set in hermaphroditic plants: studies with the Australia Proteaceae. Trends in Ecology and Evolution 4, 267-272.

Bond, P. & Goldblatt, P. (1984). Plants of the Cape flora: A Descriptive Catalogue. Journal of South African Botany, Supplementary Volume No. 13.

Bond, W.J. (1984). Fire survival of Cape Proteaceae - influence of fire season and predators. Vegetatio 56, 65-74.

Bond, W.J. (1988). Protea as "tumbleseeds: Wind dispersal through the air and soil. South african Journal of Botany 54, 455-460.

Bond, W.J. & Richardson, D.M. (1990). What can we learn from extinctions and invasions about the effects of climate change? South African Journal of Science 86, 429-433.

Bond, W.J., Vlok, J. & Viviers,M. (1984). Variation in seedling recruitment of Cape Proteaceae after fire. Journal of Ecology 72, 209 - 221.

Brits, G.J. (1986). The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes. South African Journal of Botany 52, 291-293.

Brown, J.C. & Jolley, V.D. (1989). Plant metabolic responses to iron- deficiency stress. BioScience 39, 546-551.

Chesson, P & Huntly, N. (1989). Short-term instabilities and long-term community dynamics. Trends in Ecology and Evolution 4, 293-298.

Coetzee, J.H. & Giliomee, J.H. (1987). Seed predation and survival in the infructescences of Protea repens (Proteaceae). South African Journal of Botany 53, 61-64.

Cowling, R.M. (1989). Flames and flowers: challenges of the Cape flora. South African Journal of Science 85, 289 - 290.

Cowling, R.M. (1990).Diversity components in a species-rich area of the Cape Floristic Region. Journal of Vegetation Science 1, 699-710.

Cowling, R.M., Lamont, B.B. & Pierce, S.M. (1986). Seed bank dynamics of four co-occurring Banksia species. Journal of Ecology 75, 289 - 302.

Esler, K.J., Cowling, R.M., Witkowski, E.T.F. & Mustart, P.J. (1989). Reproductive traits and accumulation of nitrogen and phosphorus during the development of fruits of Protea compacta R.Br. (Calcifuge) and Protea obtusifolia Beuk. ex Meisn. (Calcicole). New Phytologist 112, 109-115.

Fagerström, T. (1988). Lotteries in communities of sessile organisms. Trends in Ecology and Evolution 3, 303- 306.

Fagerström, T. & Agren, G.I. (1979). Theory for co-existence of species differing in regeneration properties. Oikos 33, 1-10.

Fowler, N.L. (1988). What is a safe site?: neighbor, litter, germination date, and patch effects. Ecology 69, 947-961.

Frost, P.G.H. (1984). The responses and survival of organisms in fire-prone environments. From: Booysen, P, de V. & Tainton, N.M. (Eds), Ecological Effects of Fire in South African Ecosystems , Springer Berlin.

Gentry, A.H. (1988). Changes in plant community diversity and floristic composition on environmental and geographic gradients. Annals of the Missouri Botanical Garden 75, 1-34.

Grubb, P.J. (1977). The maintainence of species-richness in plant communities: the importance of the regeneration niche. Biological Review 52, 107-145.

Hamrick, J.L. & Lee, J.M. (1987). Effect of soil surface topography and litter cover on the germination, survival, and growth of musk thistle (*Carduus nutans*). *American Journal of Botany* 74, 451-457.

Harper, J.L. & Chancellor , A.P. (1959). In: Harper ,J.L. (1977). *Population Biology of Plants.* Academic Press, London.

Harper, J.L., Williams, J.T. & Sagar, G.R. (1965). The behaviour of seeds in the soil. I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. *Journal of Ecology* 53, 273-286.

Jongens-Roberts, S.M. & Mitchell, D.T. (1986). The distribution of dry mass in an evergreen fynbos shrub species *Leucospermum parile* (Salisbury ex J.Knight) Sweet (Proteaceae), at different stages of development. *New Phytologist* 103, 669-683.

Jordaan. P.G. (1949). Aantekeninge oor die voortplanting en brandperiodes van *Protea mellifera* Thunb. *Journal of South African Botany* 15, 121-125.

Kruger, F.J. & Taylor, H.C. (1979). Plant species diversity in Cape Fynbos: gamma and delta diversity. *Vegetatio* 41, 85-93.

Lamont ,B.B., & Barker, M.J. 1988. Seed bank dynamics of a serotinous fire-sensitive *Banksia* Species. *Australian Journal* of Botany 36, 193 - 203.

Lamont, B.L., Collins, B.G. & Cowling, R.M. (1985). Reproductive biology of the Proteaceae in Australia and South Africa. Proceedings of the Ecological Society of Australia 14, 213-224.

Lee, W.G. & Fenner, M. (1989). Mineral nutrient allocation in seeds and shoots of twelve Chionochloa species in relation to fertlility. Journal of Ecology 77, 704-716.

Lamont, B. B., Hopkins, A.J.M. & & Hnatiuk, R.J. (1984). The flora composition, diversity and origins. From: Pate, J.S. & Beard, J.S. (Eds). Kwongan, Plant Life of the Sand Plain. University of Western Australia Press, Nedlands.

Linder, H.P. (1985). Gene flow, speciation, and species diversity patterns in a species-rich area: the Cape flora. From: Vrba, E.S. (Ed.), Species and Speciation, Transvaal Museum Monograph No. 4. Transvaal Museum, Pretoria.

Manders, P.T. (1986). Seed dispersal and seedling recruitment in Protea laurifolia. South African Journal of Science 52, 421-424.

Mayer, A.M. & Poljakoff-Mayber, A. (1975). The Germination of Seeds. Pergamon Press.

Mazer, S.J. (1989). Ecological, taxonomic, and life history correlates of seed mass among Indiana dune angiosperms. Ecological Monographs 59, 153-175.

Midgley, J.J. (1988). Mortality of Cape Proteaceae seedlings during their first summer. South African Forestry Journal 145, 9-12.

Midgley, J.J. (1989). Season of burn of serotinous Proteaceae: A critical review and further data. South African Journal of Botany 55, 165 - 170.

Pate, J.S., Rasins, E., Rullo, J. & Kuo, J. (1985). Seed nutrient reserves of Proteaceae with special reference to protein bodies and their inclusions. Annals of Botany 57, 747-750.

Silvertown, J. (1987). Introduction to Plant Population Ecology. Longman Scientific and Technical.

Silvertown, J.L. (1989). The paradox of seed size and adaptation. Trends in Ecology and Evolution 4, 24-26.

Tyson, P.D. (1991). Climate change in Southern Africa. Past and present conditions and future scenarios. Climatic Change 18, 241-258.

van Staden, J. & Brown, N.A.C. (1977). Studies on the germination of South African Proteaceae - a review. Seed Science and Technology 5, 633-643.

van Wilgen, B.W. & Viviers, M. (1985). The effects of season of fire on serotinous Proteaceae and the implcations for fynbos management. South African Forestry Journal 133, 49 - 53.