Propagation of Cape Proteaceae, Ericaceae, and *Restionaceae* from Seed

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The Cape Floral Region possesses the richest temperate flora in the world. The dominant vegetation of the Cape Floral Region is the fynbos which is typified by the presence of members of the Restionaceae (Cape reeds and grasses), the Proteaceae (sugarbushes, pincushions, and conebushes), Ericaceae (Cape heaths), and a number of endemic families. Many of the fynbos species are of outstanding horticultural potential. Seeds of most species are dormant and research has shown that very specific environmental cues are required for germination. It has also been shown that fire, a natural feature of the fynbos environment, provides the major cues for seed germination in the wild. During the last five years, considerable progress has been achieved in understanding seed dormancy mechanisms in the Proteaceae, Ericaceae, and Restionaceae.

INTRODUCTION

Fynbos is a unique type of vegetation which is dominant in the Cape Floral Region (CFR) in the south western Cape, at the southern tip of Africa. The CFR covers an area of 90,000 km^2 (35,000 sq miles), which is less than 4% of the area of South Africa, yet it contains 8600 plant species and is by far the richest temperate flora in the world. Over two-thirds of the Cape plant species and seven of the plant families are endemics. Fynbos, which is a community of small shrubs, evergreen and herbaceous plants, and bulbs, is exceptionally rich in species and contributes most of the species to the flora of the CFR. It is perhaps best known as the home of the South African proteas (sugarbushes, pincushions, and conebushes) and *Erica* (Cape heaths) and is also typified by the Restionaceae (Cape reeds or Cape grasses) (Brown et al., 1995).

Many of the species from these fynbos families are cultivated as ornamentals in parks and gardens around the world, or are of importance as floricultural crops. Propagation of fynbos plants from seed is difficult as seeds of many species are dormant when shed and require very specific environmental "messages" or cues before they will germinate (Brown, 1993). The fynbos occurs in areas with a Mediterranean climate (winter rainfall), and the environment is characterised by a number of stress factors such as summer drought, low soil fertility and periodic fires. The fires have a frequency of 5 to 40 years and are a natural phenomenon in fynbos. Seeds of many species are adapted to germinate in response to one or more of the cues provided by fire. Heat from flames may fracture the impermeable seed coat of hard-seeded species (e.g. Fabaceae) resulting in the coats becoming permeable to water. Dry heat may also break dormancy by providing a heat-pulse which stimulates the embryo directly and results in germination (e.g. Restionaceae). Dry heat has also been reported to break seed dormancy of some South African *Leucospermum* (pincushions, family Proteaceae) by complete desiccation of their oxygen-impermeable seed-coats. When rain falls the dry coats, which are permeable to water, split suddenly and the embryo can then obtain sufficient oxygen for germination. Fires also provide chemical messages or cues, such as the gases ethylene and ammonia, which stimulate germination in some species of *Erica*. In addition to the more obvious cues provided by heat, it has recently been discovered that smoke from fynbos fires provides a major (yet unidentified) chemical cue which is responsible for stimulating the germination of seed of many fynbos species (Brown, 1993; Van Staden et al., 1995). Fire may also have an indirect effect on germination by causing changes in the soil temperature regimes in the immediate post-fire environment (Brown, 1993). Fire thus provides the major cues for germination in the wild and these cues have to be simulated when attempting to germinate seed in the laboratory and nursery.

SEED GERMINATION STUDIES

Cape Proteaceae. The seed biology of the family Proteaceae, with approximately 300 members, has been the most extensively researched and these findings have been reviewed by Van Staden and Brown (1977) and Brits (1996). The Cape Proteaceae have two distinct achene types. One type is rounded (often ellipsoid) relatively hard and nut-like and is stored in the soil. Germination is characterised by the splitting of the seed coat due to cotyledon expansion, which is then followed by protrusion of the radicle. In the second type, the achene is winged, plumed or hairy (often flattened), and relatively soft. The latter type is produced mostly by serotinous species, i.e. species in which seeds are stored in the living plant canopy. In serotinous species germination is first indicated by penetration of the seed coat by the radicle. Serotinous genera include *Protea*, *Aulax*, and most of the *Leucadendron* and make up approximately 37% of the Cape Proteaceae. The remainder, excluding *Brabejum*, being nut-like. Nut-like and serotinous achenes show different germination syndromes.

Proteaceae With Nut-like Achenes. These achenes do not germinate or germinate poorly in mature fynbos vegetation, but seedlings recruit en masse during the first winter after fire. The breaking of dormancy in species with nut-like achenes is strongly dependent on moderately low seasonal air temperature. This is not a stratification requirement but the low temperature requirement is a mechanism to promote germination during the favourable cool, moist western Cape winter period. High diurnal temperature is also required for maximum germination. A range of fluctuating temperatures is equally effective in stimulating germination, e.g. 4 to 10C (night) and 20 to 28C (day). Seed-coat-imposed dormancy by means of oxygen exclusion is a characteristic of most Proteaceae with nut-like fruits (10 out of 14 species of three genera tested), but not of serotinous species (13 out of 15 species of three genera tested). Germination of achenes of *Leucospermum* species can be improved by a single 24-h treatment with relatively low concentrations (0.01 to 0.1%) of hydrogen peroxide. A relatively slight increase in the level of oxygen available to embryos is usually sufficient to initiate germination under suitable environmental conditions (Brits, 1996).

Recommendations

- The intact seed coat is readily permeable to water but poorly permeable to oxygen. To improve oxygenation, soak seeds in 1% H_2O_2 before making commercial sowings in seed beds. In the laboratory, incubate seeds in oxygen.
- Seed germination is strongly dependent on seasonal low temperature. Therefore, sow seeds in seed beds during autumn or early winter. In the laboratory, incubate seeds at an optimum low temperature of 8 or 9C.
- High temperature is also required for germination and should be alternated with low temperature on a daily basis. Commercial seed beds should thus be constructed in full sun. In the laboratory the optimum high temperature of 24C should be maintained for 8 h per day followed by a period of low night temperature (16 h).

Serotinous Proteaceae. Serotiny is an adaptive response to cyclical fire in fynbos. In nature, seeds are shed following a fire and germinate en masse only after fire. Seeds have a low temperature requirement for germination (1 to 11C) which allows the avoidance of drought by synchronising germination with the first (wet) winter season following dispersal. In contrast to nut-fruited species, the pericarp in serotinous species apparently plays a lesser role in preventing oxygen diffusion to the embryo.

Recommendations:

- Use freshly harvested seed, as seeds lose viability with age.
- Germinate seeds at temperatures below 20C, preferably between 1 to 11C.
- If seeds are of uncertain age and viability and/or incubation temperatures are above 20C, these factors may be counteracted by presoaking seeds in a solution containing GA_3 or GA_4 and GA_7 (Brown and Drewes, 1991).
- Sow seeds in a well-aerated, well-drained, sandy soil and avoid waterlogging.

Cape Ericaceae - Ericoideae. Seed germination studies in this family have recently been reviewed by Brown et al. (1993). Ninety-five percent of the 857 species are confined to the southern tip of Africa and many are of importance in horticulture and floristry. Fire is very important in the ecology of the Ericoideae and the vast majority of species regenerate only from seed after a veld fire. Seeds are very small and are, in all but one species, shed when ripe. Serotiny is rare in this family and is found only in *Erica sessiliflora*.

Factors Of Importance In Germination.

1) Germination may be stimulated by dry heat and the gases ethylene and ammonia.

2) Germination is stimulated by soaking seeds in GA_3 or GA_4 and GA_7.

3) Alternating day/night temperatures, as occur during winter in burnt fynbos, are an important cue for germination.

4) Germination is stimulated by plant-derived smoke and aqueous smoke extracts. In the first major gemination study in this family, Brown et al. (1993), screened seed of 40 species to obtain an indication of how important the smoke cue was for germination. The improved germination following smoke treatment shown by 26 of the 40 species tested, suggested that under natural conditions smoke from fynbos fires provided an important cue for triggering seed germination in this family. It was also suggested that the nine species which showed a 1000% or more increase in germination following smoke treatment formed a group in which smoke was likely to be the major cue for germination. In those species in which there was a lesser response, smoke might be one of a number of cues which include heat and alternating high and low incubation temperatures. Amongst the species responding to smoke treatment were a number of species of particular horticultural importance. The smoke treatment ensures a much greater efficiency when propagating from seed and this is of importance in plant breeding programmes. It should also make more plants available to the horticulture industry.

Recommendations:

- Use fresh mature seed.
- Soak seeds in aqueous smoke solution for 24 h before sowing, or "smoke" seeds sown in seed trays. The trays should have a sand, loam, and bark mixture and be well drained.
- Alternatively, seeds may be pre-soaked in GA_3 or GA_4 and GA_7 solution prior to sowing.
- Incubate seeds under alternating night/day temperatures, e.g. 10C for 16 h per night; 15 to 25C for 8 h per day.

Cape Restionaceae. The Restionaceae is a family of evergreen, rush-like plants which is almost restricted to the southern hemisphere. There are about 320 species in Africa, (300 in Cape) and 100 in Australia. The African Restionaceae are relatively diverse in their seed dispersal mechanisms, which could be implicated in the survival of seeds during or after fires. The modes are: (1) wind dispersal of unilocular, indehiscent ovaries, with a persistent perianth which acts as a wing for the fruit; (2) myrmecochory of fruits containing elaisomes. The ovary is unilocular and indehiscent, and the ovary wall is heavily lignified (e.g. the "nut-fruited" restiads). These seeds are also serotinous and are retained on the plant until the next season's seed crop is mature; and (3) the so-called "basic" condition, showing dehiscent ovaries, with 1 to 3 locules. Here the seed is released from the ovary after maturation, but it is not known how it is dispersed after release (Brown et al., 1994).

Factors of Importance in Germination. The poor germination achieved with seed of many species has been attributed to the limited seed set of some species and the difficulty in determining when seeds are mature and ready for harvest.

1) Heat treatment of seeds at 120C for 3 min gave a significant improvement in the germination of seeds of some species.

2) In common with many other fynbos species, Restionaceaes require alternating high and low diurnal temperatures as a cue for germination.

3) Germination is stimulated by plant-derived smoke and aqueous smoke extracts. Brown et al. (1994) conducted a major study in which seed of 32 species were screened to obtain an indication of how important the smoke cue is for germination in this family. The results of this study represented the first occasion that comparative germination data for South African species had ever been obtained. Twenty-five of the 32 species tested showed a statistically significant improvement in germination following smoke treatment. Untreated seeds of 18 of the species responding showed a high degree of dormancy with only 0.1% to 2.0% germination. These results suggested that under natural conditions smoke from fynbos fires provided an important cue for triggering seed germination in this family. It was also suggested that the 16 species which showed a 1000% or more increase in germination following smoke treatment, formed a group in which smoke was likely to be the major cue for germination. In those species in which there is a lesser response, smoke is probably one of a number of cues which include heat, and alternating diurnal high and low incubation temperatures. The four species that did not germinate were all myrmecochorus, nut-fruited species, which possibly require a different or additional heat cue for germination.

Recommendations

- Use fresh mature seed.
- Seeds may be pre-soaked in aqueous smoke extract for 24 h before sowing; or seeds may be smoked once sown in trays. Fill trays with a sand, loam, and bark mixture which is well drained.
- Incubate seeds under alternating night/day temperatures of 8C for 16 h and 28C for 8 h for optimum germination.
- Nut-fruited species should be heated to 120C for 3 min prior to pre-soaking in aqueous smoke extract. Germination cues for nut-fruited restios require further study.

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