South African Journal of Botany 92 (2014) 112–119

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Alternating temperature requirements in *Leucospermum* R.Br. seed germination and ecological correlates in fynbos

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ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form 31 January 2014
Accepted 19 February 2014
Available online 25 March 2014

Edited by MI Daws

Keywords:
Adaptive response
Alternating temperature
Ecophysiology
Fynbos ecology
Leucospermum
Proteaceae
Persistent seed bank
Seed dormancy
Seed germination

ABSTRACT

The effect of germination incubation regimes, which were chosen on the basis of temperature patterns found within the fynbos (Mediterranean fire-prone shrubland-type) habitat in the Cape Floristic Region during the autumn germinative period, was tested on seeds of 6 *Leucospermum* species over 5 taxonomic sections (*L. cordifolium*, *L. cuneiforme*, *L. erubescens*, *L. glabrum*, *L. reflexum*, *L. vestitum*). Seeds of each species were scarified, soaked in water and incubated at 15 combinations of diurnal low (16 h) and high (8 h) temperatures. The results indicated that *Leucospermum* seeds generally require alternating temperatures for germination. Regression analysis of germination percentage responses were used to estimate provisional optimum low [$T_O(LOW)$] and optimum high [$T_O(HIGH)$] temperature requirements for individual species. These requirements differed markedly among species, and across species they averaged 9.9 and 21.2 °C, respectively. Individual requirements correlated positively with mean late autumn air temperatures obtained from weather stations near the natural habitats of species. We conclude that a diurnal alternating temperature requirement is a character syndrome in *Leucospermum* in which the $T_O(LOW)$ and $T_O(HIGH)$ components are narrowly adaptive, independent and genetically stable characters relating to the known ecophysiological roles of low and high temperature requirements (Brits, Cutting, Brown and Van Staden, 1995). Ecological and adaptive roles of these requirements are compared with those in some persistent small-seeded soil-stored seed banks.

1. Introduction

A requirement for a diurnally alternating temperature is a major factor in the germination ecophysiology of wild (non-domesticated) seeds (Probert, 1992). The subject has consequently become well-researched (Baskin and Baskin, 2001; Morinaga, 1926; Murdoch et al., 1989; Thompson, 1974; Thompson and Grime, 1983). The approach in this study field has often been limited to the quantification of temperature requirements with few attempts to correlate thermoperiodicity with underlying physiological processes (e.g. da Silva et al., 2007; Murdoch et al., 1989). However, correlation of alternating temperature requirements with ecological determinants in the natural recruitment of species has been attempted. Examples in the literature of proposed ecological function related to increased temperature fluctuation, are: a depth sensing mechanism enabling superficially deposited seeds to emerge (Probert, 1992; Thompson and Grime, 1983); a mechanism to avoid flooding of seedlings (Thompson, 1974; Thompson and Grime, 1983); and a means of ensuring that a competing vegetation canopy has been removed, for example after the creation of tropical forest canopy gaps (Pearson et al., 2002; Vazques-Yanes and Orozco-Segovia, 1982).

Some species of fire-prone habitats in Mediterranean-type climates avoid competition, by detecting the occasional removal of a dense vegetation canopy by fire, by means of alternating temperature requirement. The soil-stored seeds of nut-fruited Proteaceae in the fynbos of the Cape Floristic Region, for example of *Leucospermum* R.Br., respond to the favorable post-fire condition via a requirement for increased daily soil temperature fluctuation (Brits, 1987).

Leucospermum cordifolium (Salisb. ex Knight) Fourcade and *Serruria florida* Knight (another nut-fruited fynbos Proteaceae species) seeds germinated maximally (final germination percentage response) when incubated under alternating moderately low and high temperatures within a 24 h period, i.e. paralleling natural night- and daytime temperatures within the range of 7–11 °C and 20–25 °C and a slightly cooler optimal regime was indicated for *S. florida* than for *L. cordifolium* (Brits, 1986). Both species have relatively narrow ecological ranges (Rourke, 1972; Vogts, 1982), however *S. florida* grows at a higher, cooler altitude. Experimental individual requirements of these species thus

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http://dx.doi.org/10.1016/j.sajb.2014.02.008
0254-6299/

appeared to be correlated with differences in the climatic temperature regimes associated with their mountainous habitats.

The natural Cape fynbos habitat of *Leucospermum* species is characterized by contrasting microclimates (Rourke, 1972) and the question arises whether, and how closely, species are adapted to their environmental temperature regimes.

Leucospermum seed germination involves the interaction of environmental and seed factors, including several forms of innate dormancy as well as natural variation in viability across seed lots (Brits et al., 1999; Kelly et al., 1992). To distinguish between poor viability and dormancy effects we employed viability testing of seed lots with tetrazolium in the present study (Brits and Van Niekerk, 1976; Brits, 1990; Brits et al., 1999).

In previous work, the six species chosen for this study (Table 4) germinated maximally when incubated under an alternating temperature regime, in combination with oxygenation related treatments (Brits, 1990). Assuming that natural scarification of *Leucospermum* seeds must occur in the soil-stored seed bank before oxygen dependent germination can take place (Brits, 1990; Brits et al., 1993, 1999) we minimized possible confounding effects of temperature with the dormancy effects of intact (oxygen impermeable) seed coat layers in our study, and also simulated natural scarification in fynbos, by applying concentrated H_2SO_4 acid scarification prior to incubation (Brits and Van Niekerk, 1976; Brits et al., 1999). However, the status of smoke as a ubiquitous germination stimulant is as yet inconclusive in *Leucospermum* (Brown and Botha, 2004) and no other seed dormancy factors, such as light requirement (Van Staden, 1966) are known to play a role.

Germination rate is important for interpreting the dynamics of ecology, for example the capacity of dispersed seed to spread germination through time (Ranal and de Santana, 2006). In *Leucospermum* cultivation seed dormancy may manifest as slow or erratic germination (Vogts, 1982) and we therefore also studied the response of germination rate (GR) to temperature treatments.

Our chosen temperature incubation range followed previous work with *L. cordifolium* (Brits, 1986) and encompassed the average minimum and maximum air temperatures recorded across the respective habitats of species during the autumn germination period (Table 4). The choice of a single 16 h low × 8 h high diurnal temperature cycle was based on the ambient temperature regimes prevailing in fynbos during autumn. This is in accordance with the need for relatively long periods at low temperature for optimal germination response within diurnally fluctuating temperature cycles (Thompson, 1974; Totterdell and Roberts, 1980).

Seeds in this, and previous studies were obtained from essentially non-domesticated sources, usually produced within fynbos areas close to their natural habitats, or collected directly from the wild (e.g. Brits, 1990 — except *L. cordifolium*, see the Materials and Methods section). Experience has shown that *Leucospermum* seed characters are highly heritable and that present-day seeds behave essentially as wild-type material.

The present study tests three hypotheses relating to germination temperature requirements in a group of 6 *Leucospermum* species with contrasting phylogeny (representing 5 of the 9 taxonomic sections in the genus — Table 4) and originating from varied climatic backgrounds: 1) that diurnally alternating temperatures are correlated requirements within the genus in fynbos (character syndromes sensu Angevine and Chabot, 1979); 2) that the temperature requirements of individual species differ; and 3) that specific differences are correlated with ecological determinants, i.e. they are a function of selective pressures.

2. Materials and methods

2.1. Seed sources

Leucospermum seed was produced under cultivation at the Tygerhoek Experimental Farm at Riviersonderend, South Africa (34°9′S, 19°54′E) within a typically mesic mountain fynbos area. Fresh, dry, fully matured seeds were harvested under controlled conditions and hand sorted, and in a few instances of short supply they were supplemented by cultivated 1–2 yr-old seed obtained from commercial seed suppliers. All experimental seed plants had been cultivated for only one or two generations from seed following collection from the wild (Brits, 1990) excepting *L. cordifolium*, a partially domesticated species ("wild/crop species" sensu Watt and Bloomberg, 2012).

Seeds were thoroughly mixed and divided into lots of convenient size for acid scarification treatment. Seeds were treated for 7 min in (c) H_2SO_4 (based on previous tests with *L. cordifolium* — Brits and Van Niekerk, 1976), washed and soaked in distilled water for 24 h. Seeds were then air-dried and dusted with thiram wp fungicide (Brits, 1990). Petri dishes (9 cm) were divided into 6 segments and 25 seeds per species (except 22 for *L. cuneiforme* and *L. erubescens*) were transferred to each segment per petri dish onto one layer of Whatman no. 1 filter paper. Each petri dish constituted one replication and this was repeated 6 times within each of the temperature regimes.

2.2. Temperature regimes

Seeds were dark-incubated at 15 different constant and alternating temperature regimes (Table 1). Where temperatures were alternated the lower and higher temperatures were maintained for 16 and 8 h respectively. Mean daily temperature was calculated as the time weighted mean of the two temperatures.

Alternating temperatures were obtained by transferring petri dishes with seeds from one to another of five incubators which were kept at constant temperatures, with a daily variation not exceeding ±1 °C. A seed was considered to have germinated when the radicle had emerged 1 mm; germinated seeds were counted and removed weekly for 14 weeks, giving both cumulative and final germination percentage responses.

A viability test with tetrazolium (Brits and Van Niekerk, 1976) was carried out on samples of all 6 species.

The relationship between mean daily temperature, temperature amplitude and germination percentage was determined by means of bivariate 2nd order polynomial regression analysis. From these the turning points (maxima) were estimated of X_1 (mean daily temperature) and X_2 (temperature amplitude) for untransformed percentage data. Using the estimated turning points X_1' and X_2', the optimum diurnal low [$T_O(LOW)$] and optimum high [$T_O(HIGH)$] germination temperatures (for eliciting maximal germination percentage responses) for the six species were calculated by transposition (Brits, 1986):

$$T_O(LOW) = X_1' - 1/3X_2'$$

and

$$T_O(HIGH) = X_1' + 2/3X_2'.$$

Table 1
Incubation temperature regimes (°C), their means and amplitudes, for six experimental *Leucospermum* species.

	Regime	Mean	Amplitude		Regime	Mean	Amplitude
1.	4	4	0	9.	4 × 28	12	24
2.	10	10	0	10.	10 × 16	12	6
3.	16	16	0	11.	10 × 22	14	12
4.	22	22	0	12.	10 × 28	16	18
5.	28	28	0	13.	16 × 22	18	6
6.	4 × 10	6	6	14.	16 × 28	20	12
7.	4 × 16	8	12	15.	22 × 28	24	6
8.	4 × 22	10	18				