

Seeds Use Temperature Cues to Ensure Germination under Nurse-plant Shade in Xeric Kalahari Savannah

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- **Background and Aims** In arid environments many plant species are found associated with the canopies of woody perennials. Favourable conditions for establishment under canopies are likely to be associated with shade, but under canopies shade is distributed patchily and differs in quality. Diurnal temperature fluctuations and maximum temperatures could be reliable indicators of safe sites. Here, an examination is made as to whether canopy-associated species use temperature cues to germinate in shade patches, rather than matrix areas between trees.
- **Methods** The study was carried out in arid southern Kalahari savannah (Republic of South Africa). Perennial and annual species associated with *Acacia erioloba* trees and matrix species were germinated at temperature regimes resembling shaded and unshaded conditions. Soil temperature was measured in the field.
- **Key Results** Germination of all fleshy-fruited perennial acacia-associated species and two annual acacia-associated species was inhibited by the temperature regime resembling unshaded conditions compared with at least one of the regimes resembling shaded conditions. Inhibition in perennials decreased with seed mass, probably reflecting that smaller seedlings are more vulnerable to drought. Germination of matrix species was not inhibited by the unshaded temperature regime and in several cases it increased germination compared with shaded temperature regimes or constant temperature. Using phylogenetically independent contrasts a significant positive relationship was found between canopy association and the germination at shade temperatures relative to unshaded temperatures.
- **Conclusions** The data support the hypothesis that canopy species have developed mechanisms to prevent germination in open sun conditions. The results and data from the literature show that inhibition of germination at temperature regimes characteristic of open sun conditions can be found in fleshy-fruited species of widely divergent taxonomic groups. It is predicted that germination mechanisms to detect canopy shade based on temperature cues are widespread in species depending on nurse plants, especially bird-dispersed species.

Key words: Directed dispersal, facilitation, nurse plants, germination cue, daily temperature fluctuation, maximum temperature, fleshy-fruited plants, ornithochory, Kalahari, arid environments.

INTRODUCTION

In vegetation characterized by discontinuous tree or shrub layers a distinct assemblage of plant species ranging from annuals to trees is commonly associated with the canopies of the initially establishing pioneer woody species. Such patterns are most commonly found in arid regions (Went, 1942; Archer *et al.*, 1988; Pugnaire *et al.*, 1996; Facelli and Brock, 2000) where facilitation is most important (Callaway and Walker, 1997; Holmgren *et al.*, 1997; Flores and Jurado, 2003), but can also be found in several other environments (reviewed in Flores and Jurado, 2003). The establishment of many species in arid areas depends completely on their so-called nurse plants, a phenomenon which has been especially well studied for Cactaceae (Steenberg and Lowe, 1969; Valiente-Banuet and Ezcurra, 1991; Valiente-Banuet *et al.*, 1991; Godínez-Alvarez *et al.*, 2002). In arid environments local conditions, like temperature, soil moisture, soil nutrients and radiation are different under canopies compared with open spaces (Belsky *et al.*, 1989, 1993; Weltzin and Coughenour, 1990; Vetaas, 1992; Belsky and Canham, 1994). Experimental studies have shown that some of these

conditions under the canopy, such as shading and higher nutrient levels, improve survival and growth of seedlings under the canopy compared with open spaces (Valiente-Banuet and Ezcurra, 1991; Carillo-García *et al.*, 2000).

At the same time dispersal probably plays an important role in the development of such associations. Many of the perennial species associated with canopies are bird dispersed (McDonnell and Stiles, 1983; Tester *et al.*, 1987; Dean *et al.*, 1999; Facelli and Brock, 2000), and the seed rain of bird-dispersed species tends to be concentrated under isolated trees used by birds for perching, roosting and nesting (McDonnell and Stiles, 1983; Tester *et al.*, 1987; Hoppes, 1988; Izhaki *et al.*, 1991; Guevara and Laborde, 1993; Debussche and Isenmann, 1994; Holl, 1998). For annuals, epizoochory and endozoochory by large herbivores that rest and defaecate under trees while seeking shade has been suggested as a cause for this pattern (Janzen, 1984; Milton and Dean, 1995).

When both the seed rain and the optimal conditions for establishment show a patchy distribution and are spatially correlated one can speak of directed dispersal (Howe and Smallwood, 1982). Wenny (2001) hypothesized that directed dispersal is especially common in arid regions. It can be expected that in canopy-associated species in arid environments directed dispersal, i.e. an interaction

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between dispersal pattern and favourable local conditions, has led to the development of germination responses to detect these conditions. Tree canopies in savannas reduce maximum soil temperature and daily temperature fluctuations (DTF) (Tiedemann and Klemmedson, 1977; Belsky *et al.*, 1989). These temperature parameters under canopies are likely to be highly correlated with favourable conditions for establishment and growth like reduced daytime temperatures, reduced radiation and higher soil moisture. Temperature fluctuation has long been recognized as a cue that seeds use for gap detection (Thompson and Grime, 1983; Bullock, 2000). Germination of gap-colonizing species in tropical rain forests, for example, is stimulated by increased temperature fluctuation typical of gaps (Vázquez-Yanes and Orozco-Segovia, 1982; Pearson *et al.*, 2002). Likewise canopy specialists could use temperature cues to detect a canopy patch. In this case germination should be inhibited by high temperature fluctuations or maximum temperatures as found in open spaces.

It might seem redundant to develop a mechanism that ensures germination under canopies when directed dispersal ensures the deposition of the majority of seeds under trees. But these factors are not mutually exclusive because the sub-canopy patch is not a homogeneous environment. For example, the amount of shading under the canopy differs depending on cardinal orientation (Belsky *et al.*, 1989; Belsky and Canham, 1994). Valiente-Banuet and Ezcurra (1991) found that, in Mexico, several succulents that depend on nurse plants for establishment were only found at the north side of nurse plants. However, seed rain of fleshy-fruited cacti might still be considerable on other sides due to birds perching overhead. Sapling and mature trees also differ in the shade they provide, adding further to heterogeneity in the canopy layer (Dean *et al.*, 1999). It could therefore be profitable for a seed to wait until secondary dispersal or growth of the canopy results in more favourable conditions for establishment. Animal activity in arid landscapes is often concentrated under shrubs and trees as they provide services like cover, shade and food, which increases the chance of secondary dispersal (Dean *et al.*, 1999).

It is hypothesized that canopy species have developed mechanisms to avoid germination at temperatures characteristic of open unshaded spaces in response to the heterogeneous distribution of shade favourable for establishment under the canopy. Matrix species are expected to be stimulated by temperature regimes characteristic of open conditions and inhibited by low DTF or maximum temperatures. Their mainly wind-dispersed seeds will frequently land under canopies where establishing seedlings would be out-competed by canopy species adapted to exploit high nutrient levels under shaded conditions. These hypotheses were tested with plants from the semi-arid savannah of the southern Kalahari where a distinct set of species, mainly annuals and fleshy-fruited perennials, is associated with the canopy of *Acacia erioloba* trees growing in dry river beds (Dean *et al.*, 1999).

A relationship between habitat association and germination response to temperature could be confounded by a relationship between seed mass and response to alternating

temperatures. Pearson *et al.* (2002) found for pioneer rainforest trees that small-seeded species did not use DTF as a gap detection cue, though large-seeded species did. Alternatively small-seeded species might also be more affected by DTF than large-seeded species as small seedlings are less tolerant of environmental hazards like drought (Leishman *et al.*, 2000). Therefore seed mass was also measured to evaluate its effect on germination behaviour.

In particular, the questions addressed in this paper are as follows. (a) Do soil temperature regimes differ between mature trees, saplings and matrix? (b) Do soil temperature regimes differ between different cardinal orientations under the canopy? (c) Do soil temperature regimes characteristic of open spaces inhibit germination of canopy-associated species? (d) Do soil temperature regimes characteristic of open spaces stimulate germination of matrix species? (e) Is the germination response to soil temperature dependent on seed mass?

To answer these questions, cover and presence of each species in canopy and matrix plots were recorded to enable classification of species according to their pattern of association with *Acacia erioloba*, temperature was measured in the field, seed mass was measured and germination tests were conducted in the laboratory for 29 Kalahari plants, both canopy and matrix species, at different temperature regimes characteristic of canopies or openings.

MATERIALS AND METHODS

Study area

Seeds were collected in the summer and autumn of 2001–2003, in the dry Nossob river valley, South Africa, approx. 40 km south of the Kalahari Gemsbok National Park entrance at Twee Rivieren. The vegetation can be described as very open savannah with a tree layer mainly consisting of *Acacia erioloba* and was classified by Acocks (1953) as the western form of Kalahari thornveld. Rainfall is about 200 mm year⁻¹. The rainy season lasts from about November to April, with 50% of the total falling between January and March, with a peak in March (Leistner, 1967). Mean maximum and minimum temperatures are 37.4 °C and 19.5 °C in January and 22.2 °C and 1.2 °C in July (Van Rooyen, 1984). For an extensive description of the vegetation, soil and climate, see Leistner (1967), Leistner and Werger (1973), Van Rooyen (1984) and Werger (1986).

Vegetation pattern

To be able to classify species according to their association with *Acacia erioloba* the presence and percentage cover of each plant species was determined under the canopy and in the open in May 2003. Plots were centred around ten mature *Acacia erioloba* trees (stem diameter >60 cm measured at 30 cm above ground level as this species tends to branch at a very low level). Under the canopy of each tree a 10 × 10 m plot centred around the base was established. Around each tree, in the open matrix

15–20 m from the stem, four 5 × 5 m subplots, one in each cardinal direction, were established.

Temperature measurements

To measure temperature the Thermochron iButton DS1921L-F51 (Maxim Dallas, Texas) was used. Thermobuttons were buried 1 cm deep, halfway between trunk and canopy drip-line and 10–15 m away from the canopy drip-line of five mature trees (stem diameter 96 ± 9 cm) and halfway between trunk and canopy drip-line under five saplings (stem diameter 20 ± 7 cm). For each sample unit a thermobutton was buried in each cardinal direction. Measurements were made every 20 min starting at 1800 h on 12 March 2003 and ending at 1740 h on 8 April 2003. However, as no rainfall was recorded for this period another data set, originally collected for other purposes, was used to evaluate the effect of rainfall on soil temperature. For the period 1 October 2002 to 30 December 2002 soil temperature was recorded with eight thermobuttons each buried under a different mature acacia halfway between stem and canopy drip-line at a random cardinal orientation and eight buried in the matrix at a random cardinal orientation, 10–15 m from the canopy drip-line. Measurements were made every 2 h starting at 0000 h. Rainfall data were provided by Prof. Dr A. O. E. Rasa on the farm Alpha, about 4 km from the study site. The average of three rain gauges on the farm was used.

Minimum temperature, maximum temperature and daily temperature fluctuation were determined for every 24 h from from the start of measurements. Daily temperature fluctuation was calculated as the difference between the maximum and minimum temperature within 24 h from the start of measurements. For statistical evaluation the average of all days was used.

Germination methods

Prior to germination tests seeds were stored at 5 °C. Tests were carried out within 6 months of seed collection. Seeds were germinated at day/night temperatures of 30/15 °C, 26/19 °C, 22/15 °C, 22/22 °C, with a 12 h day/night regime. Wet soils have lower maximum temperatures and DTF (Nobel and Geller, 1987) and comparison of the temperature data with rainfall showed that, after rain, maximum temperatures and DTF decreased (Fig. 1). The temperature of the soil under moist conditions allowing imbibition would be the temperature that seeds use to tune their germination. Therefore temperature regimes were chosen on the basis of observed temperatures after rainfall. DTF decreased to between 6 °C and 8 °C under canopies after rain and 7 °C was therefore chosen as the DTF resembling canopy conditions. In the matrix DTF dropped to a minimum of 13 °C but fluctuated more widely than DTF under the canopy with DTF above 20 °C also occurring several times after rain. According to the hypothesis tested in the present study, seeds of canopy species will use temperature mainly as a shade indicator under the canopy. The maximum temperatures and DTF

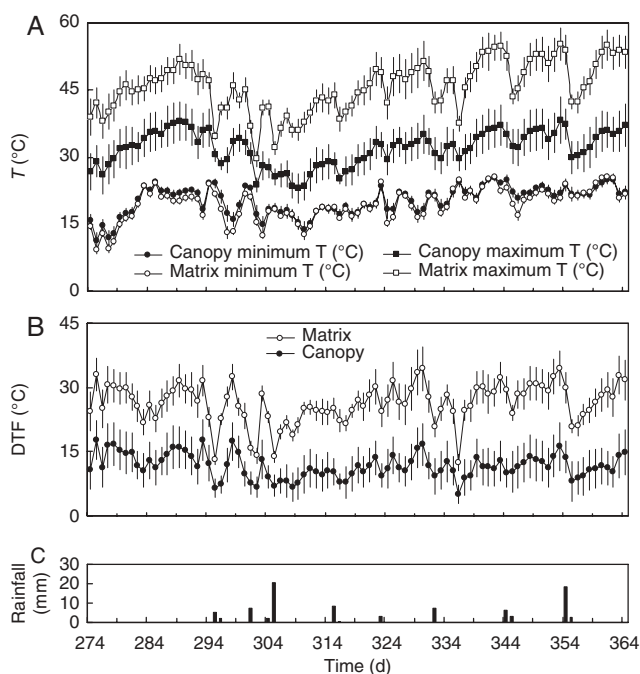


FIG. 1. Temperature parameters (mean \pm s.d., $n = 8$) and rainfall on a 24-h basis for the period 1 October 2002 to 30 December 2002. Temperature parameters based on 2-h interval measurements. (A) Maximum and minimum temperature in the matrix and under mature acacia canopies. (B) Daily temperature fluctuation in the matrix and under mature acacia canopies. (C) Rainfall. Time scale indicates day of the year.

that inhibit germination may therefore be lower than those typically found for matrix conditions. We therefore chose 15 °C to represent DTF of the soil after rain, not as an average but more as a threshold value above which canopy species should prevent germination. This was also the maximum DTF observed after rain for the most exposed measuring point under the canopy. Minimum temperature was found to differ only very slightly for different microhabitats and was somewhat arbitrarily set at 15 °C which is within the range of minimum temperatures observed after rain. Because seasonal changes in minimum and maximum temperatures could cause differences between canopy species, a 4 °C higher canopy regime was also included with DTF of 7 °C. As not enough seeds and climate chambers were available it was not possible to do the same for the matrix regime. However, as a 30/15 °C regime was found to be the optimum for germination in the majority of open land species from arid southern Africa (Veenendaal and Ernst, 1991) this was not considered critical. A constant 22 °C regime was also included.

Some species initially had a very low germinability and this was improved using dry heat treatments of 2 weeks at 50 °C (*Amaranthus dinteri*, *Amaranthus praetermissus*, annual grasses except *Aristida congesta* and *Setaria verticillata*) or 70 °C (*Aristida congesta*).

For each temperature, eight (five in *Asparagus africanus* and *Asparagus pearsonii* because fewer seeds were

available) replications of 25 seeds per Petri dish were used. For *Pentzia lanata* and *Asparagus pearsonii* the 22/15 °C is absent because not enough seeds were available. For each Petri dish two 90-mm-diameter filter paper discs (Schleicher and Schuell 595) were soaked with deionized water. Germinated seeds were counted each week until no seeds germinated any more with a maximum incubation period of 8 weeks. Finally the number of still-viable seeds was determined by pressing remaining seeds with tweezers. Firm, intact seeds were recorded as viable (Baskin and Baskin, 1998). The final value used was the percentage of viable seeds (germinated + not germinated but viable) that germinated.

Comparative method

To be able to statistically evaluate the relationship between germination response and habitat association and seed mass, indices of germination response and canopy association were calculated and seed mass was measured. An index of germination response (R) was calculated with the percentage germination at 26/19 °C (G_c) and the percentage germination at 30/15 °C (G_m) using the formula $R = (G_c/G_c + G_m)$. A value of 1 means that the species germinates only at 26/19 °C, a value of 0.5 that the species germinates equally well at both temperature regimes and a value of 0 that the species germinates only at 30/15 °C. A canopy temperature of 26/19 °C was used because this was the only temperature for which simulating regime data were available for all species. An index of canopy association (A) was calculated with the percentage cover under the canopy (C_c) and the % cover in the matrix (C_m) using $A = C_c/(C_c + C_m)$. Here a value of 1 means that the species is only found under *Acacia erioloba* canopies, a value of 0.5 that the species is equally common under canopies and in the surrounding matrix and a value of 0 that the species only occurs in the matrix. *Verbesina encelioides* was not included in this analysis because it was not found directly in the study plots and a precise measure of canopy association was therefore not available. Seed mass was determined for 20 seeds individually according to the protocol in Westoby (1998).

Because two closely related species are more likely to resemble each other on account of common ancestry than are two unrelated species, it is inappropriate to use species as independent points in statistical analysis (Harvey and Pagel, 1991). The method of phylogenetically independent contrasts (Felsenstein, 1985) was designed to use phylogenetic information to allow enumeration of the number of times relationships have evolved independently.

As a phylogeny for plant families the angiosperm phylogeny of Davies *et al.* (2004) was used. The phylogeny for the Asteraceae is based on the supertree published in Funk *et al.* (2005). For grasses, the phylogeny in Grass Phylogeny Working Group (2001) and Hilu and Alice (2001) was used. Species were assigned to their respective families and sub-families using the classification in Leistner (2000) and Gibbs Russell *et al.* (1991) for grasses.

Contrasts in germination response, seed mass and canopy association were calculated using the computer

program CAIC (Comparative Analysis by Independent Contrasts; Purvis and Rambaut, 1995) using the assumption of equal branch lengths. To meet the assumptions of normality, seed mass was \log_{10} transformed and the proportions canopy association and germination response were arcsine transformed. Contrasts were first scrutinized to see if the standardization by equal branch lengths was sufficient to render them homoscedastic, and then they were analysed using Model I multiple regression through the origin (Garland *et al.*, 1993).

RESULTS

Vegetation pattern

Cover and frequency (number of plots) for both microhabitats are given in Table 1. Species were classified as 'canopy' or 'matrix' species when their cover values were significantly associated with one of these microhabitats (Wilcoxon paired-sample test). In addition, species with no significant microhabitat association were classified as 'matrix' species, and sparsely occurring species were classified as 'canopy' species when individuals were only found beneath canopies.

Temperature measurements

For the October–December 2002 measurements (see Fig. 1) canopy and matrix were significantly different for minimum temperature (t -test for matched pairs, $t = 8.371$, $n = 91$, $P < 0.001$), maximum temperature ($t = -45.882$, $P < 0.001$) and DTF ($t = -52.505$, $P < 0.001$). Over the whole period, the average minimum temperature ranged from 11 °C to 25 °C (average 20 °C) under the canopy and from 9 °C to 26 °C (average 19 °C) in the matrix. Maximum temperature ranged from 23 °C to 38 °C (average 32 °C) under the canopy and from 30 °C to 55 °C (average 46 °C) in the matrix. DTF ranged from 5 °C to 18 °C (average 12 °C) under the canopy and from 13 °C to 34 °C (average 26 °C) in the matrix. Drops in DTF were associated with rain events. The number of days with a DTF < 10 °C was significantly positively correlated with the amount of rain ($r = 0.726$, $n = 8$, $P < 0.05$). This is important because most species growing under canopies have been found to germinate rather slowly (M. Kos, unpub. res.) and soil should remain wet and at the same time below a critical DTF for long enough to allow germination.

For the April–March 2003 data set mature trees also had significantly smaller DTFs than the matrix for all cardinal orientations (Tukey test after one-way ANOVA, for this and further results reported below, see Fig. 2A), but saplings only for the east ($P < 0.01$) and south side ($P < 0.001$) of the canopy. Saplings had significantly larger DTF under their canopies than mature trees for the north ($P < 0.01$) and east side ($P < 0.05$) of the canopy. There were no significant differences between cardinal orientations for both the matrix and mature trees. For saplings, north and south side ($P < 0.01$) and west and south side ($P < 0.05$) differed significantly in DTF. Maximum

TABLE 1. Study species with family, life form after Raunkiaer (taken from Leistner; 1967), % cover and frequency (number of plots) for ten canopy and ten matrix plots, significance level for a Wilcoxon paired-sample test for differences between canopy and matrix cover, seed mass and dispersal mode

Species	Family	Life form [†]	Canopy [‡]	Matrix [‡]		Seed mass (mg) ± s.d.	Dispersal mode
Perennial canopy species							
<i>Asparagus africanus</i> Lam.	Asparagaceae	H	0.2 (4)	0 (0)	–	14.968 ± 2.597	Birds
<i>Asparagus pearsonii</i> Kies	Asparagaceae	H	0.2 (6)	<0.1 (1)	*	13.7 ± 1.9	Birds
<i>Pollichia campestris</i> Ait.	Illecebraceae	G–H	0.2 (8)	0 (0)	**	0.361 ± 0.062	Birds
<i>Lycium bosciifolium</i> Schinz	Solanaceae	N	6.4 (10)	1.4 (3)	*	1.292 ± 0.266	Birds
<i>Solanum capense</i> L.	Solanaceae	H	0.2 (10)	<0.1 (1)	**	3.299 ± 0.514	Birds
Annual canopy species							
<i>Setaria verticillata</i> (L.) Beauv.	Poaceae	T	1.3 (9)	<0.1 (2)	**	0.423 ± 0.074	Adhesion
<i>Chenopodium opulifolium</i> Schröd. ex Koch & Ziz	Chenopodiaceae	T	0.1 (6)	0 (0)	*	0.413 ± 0.057	Unassisted, herbivore
<i>Chenopodium cf. schraderianum</i> Roem. & Schult.	Chenopodiaceae	T	–	–	– [§]	0.085 ± 0.014	Unassisted
<i>Amaranthus dinteri</i> Schinz	Amaranthaceae	T	<0.1 (3)	0 (0)	–	0.481 ± 0.070	Unassisted, herbivore
<i>Amaranthus praetermissus</i> Brenan	Amaranthaceae	T	0.5 (10)	<0.1 (1)	**	0.380 ± 0.074	Unassisted, herbivore
<i>Portulaca oleracea</i> L.	Portulacaceae	T	<0.1 (4)	0 (0)	–	0.157 ± 0.033	Ants
<i>Coronopus integrifolius</i> (DC.) Spreng.	Brassicaceae	T	–	–	– [¶]	0.324 ± 0.048	Unassisted
Perennial matrix species							
<i>Plinthus karoovicus</i> Verdoorn	Aizoaceae	Ch	0.1 (9)	0.5 (10)	**	0.139 ± 0.027	Rain
<i>Deverra denudata</i> (Viv.) Pflsterer & Podl.	Apiaceae	H	<0.1 (1)	<0.1 (1)	–	1.875 ± 0.317	Unassisted
<i>Aptosimum lineare</i> Marloth & Engl.	Scrophulariaceae	(T–)H	0 (0)	<0.1 (3)	–	0.103 ± 0.02	Unassisted
<i>Aptosimum marlothii</i> (Engl.) Hiern	Scrophulariaceae	Ch	0.5 (10)	5.2 (10)	*	0.234 ± 0.048	Unassisted
<i>Rhigozum trichotomum</i> Burch.	Bignoniaceae	N	0.2 (5)	0.6 (5)	n.s.	15.677 ± 5.323	Wind
<i>Felicia clavipilosa</i> Grau	Asteraceae	Ch	0.4 (10)	0.2 (10)	n.s.	0.102 ± 0.037	Wind
<i>Geigeria pectidea</i> (DC.) Harv.	Asteraceae	H	<0.1 (3)	<0.1 (5)	n.s.	0.383 ± 0.082	Wind
<i>Pentzia lanata</i> Hutch.	Asteraceae	Ch	<0.1 (6)	<0.1 (5)	n.s.	0.120 ± 0.018	Wind
Annual matrix species							
<i>Aristida congesta</i> Roem. & Schult	Poaceae	T(–)H	0 (0)	<0.1 (1)	– [#]	0.355 ± 0.034	Wind, adhesion
<i>Boerhavia repens</i> L.	Nyctaginaceae	T	0.1 (6)	<0.1 (2)	n.s.	2.197 ± 0.611	Adhesion
<i>Chloris virgata</i> Swartz	Poaceae	T	<0.1 (2)	0.2 (3)	–	0.331 ± 0.077	Wind
<i>Enneapogon desvauxii</i> Beauv.	Poaceae	T	0 (0)	0.1 (6)	*	0.274 ± 0.046	Wind
<i>Eragrostis porosa</i> Nees	Poaceae	T	0 (0)	<0.1 (1)	– ^{††}	0.065 ± 0.011	Unassisted
<i>Schmidia kalahariensis</i> Stent	Poaceae	T	<0.1 (1)	<0.1 (5)	–	0.615 ± 0.209	Wind
<i>Geigeria ornativa</i> O. Hoffm.	Asteraceae	T	0 (0)	<0.1 (9)	**	0.349 ± 0.049	Wind
<i>Tribulus terrestris</i> L.	Zygophyllaceae	T	<0.1 (9)	0.5 (9)	*	1.32 ± 0.41	Adhesion
<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.	Asteraceae	T	–	–	– [‡]	1.145 ± 0.116	Wind

[†]T, Therophyte; H, hemicryptophyte; G, geophyte; Ch, chamaephyte; N, nanophanerophyte.

[‡]–, Not enough pairs available for test; n.s., not significant; * $P < 0.05$; ** $P < 0.01$.

[§]Found in 15 out of 20 canopy plots but in only two of the 20 matrix plots in a nearby area in 2002.

[#]Some seedlings were found under other trees. Otherwise only found in seed bank samples, over 90 % of all seeds were found under the canopy (M. Kos & P. Poschlod, unpubl. res.).

[#]Only observed growing in the open outside the study plots.

^{††}Recorded by Weltzin & Coughenour (1990) to have higher cover in the open than under trees.

temperature showed essentially the same pattern described for DTF between habitats (see Fig. 2B). But in addition there were significant differences between the east and north side of saplings ($P < 0.05$) and the south and west side of mature trees also differed significantly ($P < 0.05$). Minimum temperature did not differ significantly between cardinal orientations for all habitats. Minimum temperatures were significantly lower in the matrix compared with canopies of sapling and mature trees for all cardinal orientations except for the south side where matrix and sapling did not differ significantly. There were no significant differences in minimum temperature between saplings and mature trees except for the south side ($P < 0.05$).

Germination tests

Differences between treatments within species were determined by one-way ANOVA followed by a Tukey test (see Fig. 3). All fleshy-fruited perennial canopy species showed reduced germination at 30/15 °C compared with 26/19 °C. *Pollichia campestris* and *Lycium bosciifolium* germinated better at 22/15 °C compared with 26/19 °C, while *Solanum capense* showed the opposite pattern. Constant temperature inhibited germination in *Lycium bosciifolium* but did not differ from 26/15 °C and/or 22/15 °C in the other fleshy-fruited species. For matrix perennials, germination rate was highest at 30/15 °C in

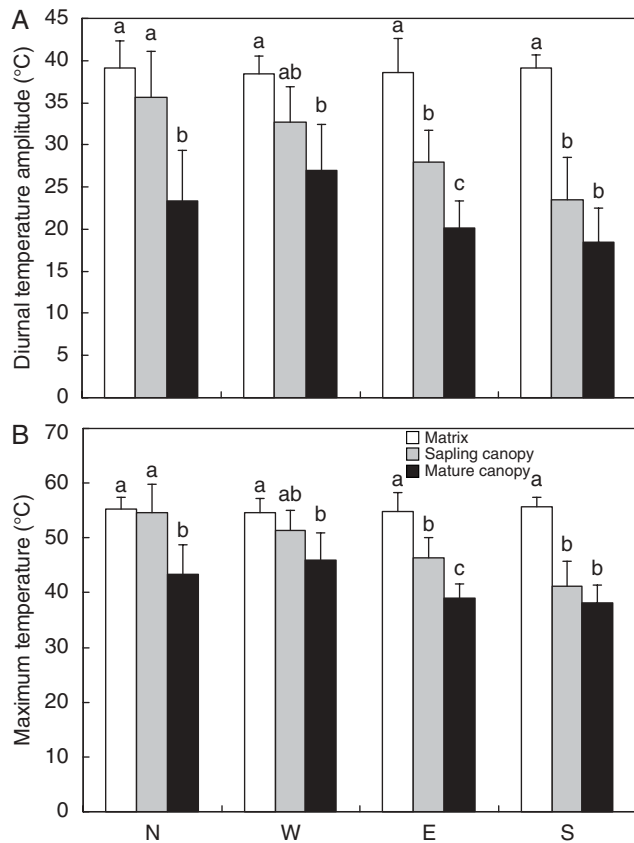


FIG. 2. Daily temperature fluctuation (A) and maximum temperature (B) in three different microhabitats for each cardinal orientation (N, north, W, west, E, east, S, south). For each combination of habitat and cardinal orientation $n = 5$. Measurements were taken 12 March to 8 April 2003. Significant differences ($P < 0.05$, Tukey test after one-way ANOVA) between habitats within one cardinal orientation are indicated by different letters. See text for details.

only one species: *Aptosimum lineare*. In *Plinthus karoocicus* both 22/15 °C and 30/15 °C produced higher germination than 26/19 °C. In the other matrix perennials no differences between treatments were found. Among the annual canopy species, *Chenopodium opulifolium* germinated better at 22/15 °C than at any other temperature but there were no differences between the other treatments, though 30/15 °C did have the lowest mean germination. *Coronopus integrifolius* germinated also better at 22/15 °C than at 26/19 °C or 30/15 °C, but germination was negligible at constant temperature. In both *Amaranthus dinteri* and *A. praetermissus* germination was stimulated by 30/15 °C compared with other treatments. No differences between treatments were found in *Chenopodium schraderianum*, *Portulaca oleraceae* and *Setaria verticillata*. Of the matrix annuals *Aristida congesta* germinated best at 30/15 °C. *Chloris virgata* had reduced germination at constant temperature. *Eragrostis porosa* showed a pattern similar to *Plinthus karoocicus*, although the difference between 26/19 °C and 30/15 °C was not significant. No differences between treatments were found for the other five matrix annuals.

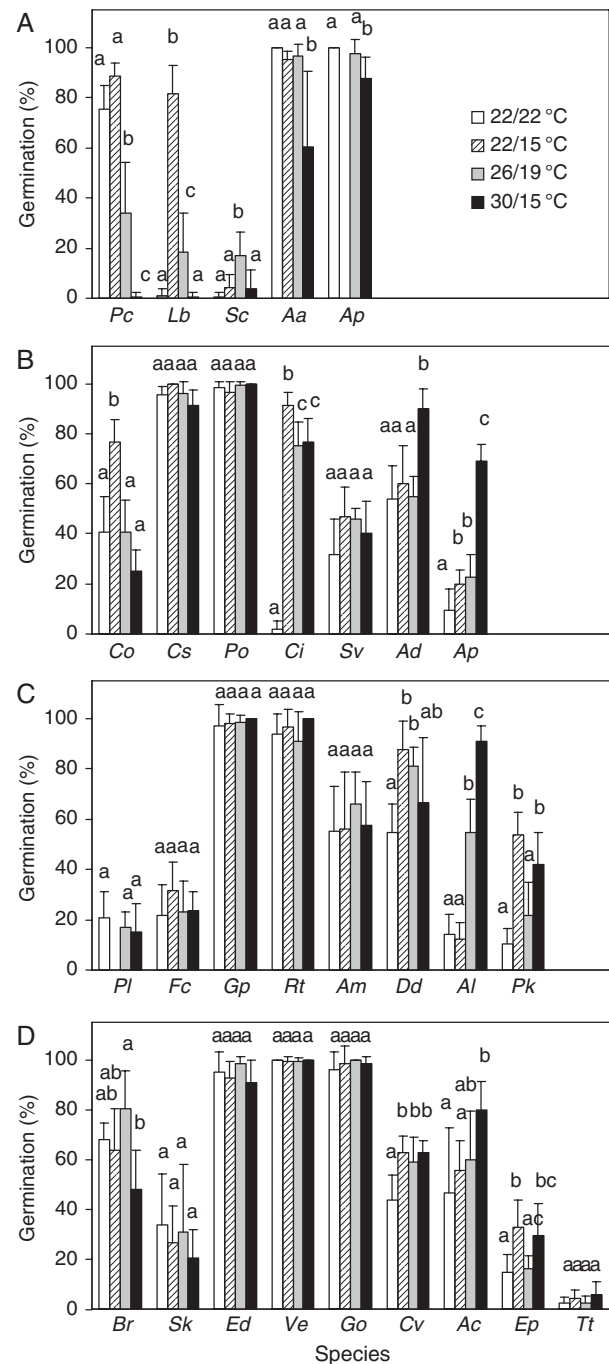


FIG. 3. Germination response (percentage of viable seeds germinating) of Kalahari plants to different temperature regimes. Significant differences between treatments within a species are indicated by different letters. (A) Canopy perennials: Pc, *Pollichia campestris*; Lb, *Lycium bosciifolium*; Sc, *Solanum capense*; Aa, *Asparagus africanus*; Ap, *Asparagus pearsonii*. (B) Canopy annuals: Co, *Chenopodium opulifolium*; Cs, *Chenopodium schraderianum*; Po, *Portulaca oleraceae*; Ci, *Coronopus integrifolius*; Sv, *Setaria verticillata*; Ad, *Amaranthus dinteri*; Ap, *Amaranthus praetermissus*. (C) Matrix perennials: Pl, *Pentzia lanata*; Fc, *Felicia clavipilosa*; Gp, *Geigeria pectidea*; Rt, *Rhigozum trichotomum*; Am, *Aptosimum marlothii*; Dd, *Deverra denudata*; Al, *Aptosimum lineare*; Pk, *Plinthus karoocicus*. (D) Matrix annuals: Br, *Boerhavia repens*; Sk, *Schmidtia kalahariensis*; Ed, *Enneapogon desvauxii*; Ve, *Verbesina encelioides*; Go, *Geigeria ornativa*; Cv, *Chloris virgata*; Ac, *Aristida congesta*; Ep, *Eragrostis porosa*; Tt, *Tribulus terrestris*.

Comparative analysis

Multiple regression models using phylogenetically independent contrasts for all species, perennials and annuals with germination response as the dependent variable and both seed mass and canopy association as independent variables were all not significant. A simple linear regression model with germination response (R) as the dependent variable and canopy association (A) as the only independent variable was significant for perennials ($\arcsin \sqrt{R} = 0.330 \times \arcsin \sqrt{A}$, $r^2 = 0.400$, $F_{1,11} = 7.327$, $n = 12$, $P < 0.05$) and all species together ($\arcsin \sqrt{R} = 0.171 \times \arcsin \sqrt{A}$, $r^2 = 0.158$, $F_{1,25} = 4.708$, $n = 26$, $P < 0.05$), but not for annuals. Linear regression models with seed mass as the only independent variable were not significant for annuals, perennials and perennials and annuals combined.

DISCUSSION

Canopy species were the only species where germination was inhibited by the matrix temperature regime. Using phylogenetically independent contrasts it was found that germination at the canopy temperature regime of 26/19 °C relative to the matrix temperature 30/15 °C increased significantly with an increase in canopy association. Comparison with temperature measurements in the field show that maximum temperatures and daily temperature fluctuation are usually well above those inhibiting or preventing germination in the lab. Even DTF and a maximum temperature marginally lower than the tested matrix regime during and after rain did not last longer than one 24-h cycle. Considering the slow germination of canopy-associated species (M. Kos, unpub. res.) this germination behaviour is very likely to prevent germination in the matrix or relatively sun exposed patches under the canopy. The temperature measurements made in the present study also show that DTF and maximum temperatures under the canopy vary strongly with cardinal orientation, which confirms the results of other studies (Belsky *et al.*, 1989; Belsky and Canham, 1994). These results support the hypothesis that maximum temperatures or DTF are used as cues to detect safe sites under the canopy in this group of plants.

No relationship was found between seed mass and germination behaviour for species or independent contrasts for perennials, annuals and all species combined. However, seed size is correlated with many aspects of plant ecology (Leishman *et al.*, 2000) and such correlations could easily obscure a relationship between the germination response to temperature and seed size. However, within the fleshy-fruited perennials, though all species are to some extent inhibited by 30/15 °C compared with 26/19 °C this inhibition decreases with seed mass. Ordering of the species according to their relative inhibition at 30/15 °C in Fig. 3 parallels a decrease in seed mass. The smallest-seeded *Pollichia campestris* showed the largest relative inhibition of germination at 30/15 °C while the *Asparagus* species still germinate to over 60 % at this temperature (Fig. 3). Pearson *et al.* (2002) found

that germination of small-seeded species of pioneer rain-forest trees (<2 mg) was unaffected by an increasing magnitude of DTF up to a species-specific threshold, above which it declined. They suggested that the negative germination response to large temperature fluctuations in small-seeded species and at extreme values for some larger-seeded species may represent a waiting strategy to avoid large-gap environments where water shortage would constrain seedling establishment. Small-seeded species are thought to be more sensitive to temperature fluctuations because they are especially susceptible to short periods of drought because of their superficial initial rooting depth (Pearson *et al.*, 2002). Thus the mechanism that gap-colonizing rain forest trees use to avoid extreme gap conditions seems to be similar to that used by acacia-associated species to avoid germination in sun-exposed sites under the canopy.

All of the fleshy-fruited canopy perennials showed inhibition of germination in matrix conditions. Within the group of annual canopy species, however, matrix temperatures had an inhibiting effect only in *Chenopodium opulifolium* and *Coronopus integrifolius*. *Boerhavia repens* also showed a significant higher germination at 26/19 °C compared with 30/15 °C. This species was classified as a matrix species because differences in cover and presence between canopy and matrix were not significant but it is the only matrix species with higher presence and cover under the canopy. The contrast between perennial and annual canopy species in their reaction to the different temperature regimes may be the result of the short life-span of annual species which makes them more flexible than perennials. In good rain years it could be possible for them to complete their life-cycle outside the canopy or at an unfavourable position under the canopy. Perennial species have to establish and grow for a longer period at the same place, which increases the need of germinating in a site where extremes over a longer period of time are avoided.

Most matrix annuals and perennials showed no differences between treatments (63 % of species), indicating that there are no strong selective forces to develop a mechanism preventing germination under canopies. Probably the chance of a seed landing in a canopy patch is small because the proportion of surface covered by tree and shrub canopies is much lower than the proportion of bare ground.

Despite the fact that many, especially fleshy-fruited, desert and savannah plants are associated with nurse-plants (Tester *et al.*, 1987; Archer *et al.*, 1988; Valiente-Banuet *et al.*, 1991) few other studies exist that tried to relate the effect of temperature on germination with an association with nurse plants (but see Fulbright *et al.*, 1995; Godínez-Alvarez and Valiente-Banuet, 1998). Fulbright *et al.* (1995) found inhibition of germination of the fleshy-fruited shrub *Celtis pallida* associated with the tree *Prosopis glandulosa* at high temperatures characteristic of interspaces whereas a species characteristic of interspaces, *Acacia smallii*, still germinated at these temperatures. They conclude that an association of *Celtis* with its nurse-plant is caused in part by dispersal by birds and in part because of inhibition of germination in interspaces.

Godínez-Alvarez and Valiente-Banuet (1998) studied the effect of DTF on germination and its relation to nurse plant associations. However, they did not find a negative effect of DTF in nurse plant-associated cacti. Moreover, some species germinated better under fluctuating temperatures than at constant temperatures. However, DTF was only 5 °C. The response may therefore be just a mechanism that prevents germination too deep in the soil. Also a positive effect of 7 °C fluctuation compared with constant temperature in *Solanum* and *Lycium* was found, but germination was inhibited at higher DTF. This is also indicated by other studies. Rojas-Aréchiga *et al.* (1997) studied the effect of light on germination of cacti. They used three different temperature regimes (25/25, 30/20 and 30/15 °C). However, the data were not analysed with regard to temperature. This probably would have revealed a decrease of germination with increasing DTF in columnar cacti as suggested by the mean values. One of the species for which the data in Rojas-Aréchiga *et al.* (1997) suggest that it germinates best at constant temperature is *Neobuxbaumia tetetzo*. This species is completely dependent on nurse plants for establishment (Valiente-Banuet and Ezcurra, 1991; Godínez-Alvarez *et al.*, 2002). Rojas-Aréchiga *et al.* (1998) propose that, based on their preference for high temperatures and light, barrel cacti may also establish in open areas whereas columnar cacti establish mainly under the shade of shrubs. In accordance with this observation Nolasco *et al.* (1997) found that in *Stenocereus thurberi*, a columnar cactus species commonly associated with nurse trees, emergence of seedlings from seeds sown in full sun was nil even when ample water was available. This suggests that modification of soil temperature by nurse plant canopies may be required for germination of this species.

It can be concluded that, for fleshy-fruited perennials, the available data from the literature support the present hypothesis that species which occur under tree canopies have developed mechanisms to prevent germination in open sun conditions. Our results and data from the literature show that inhibition of germination at temperature regimes characteristic of open spaces can be found in fleshy-fruited species of widely divergent taxonomic groups. We predict that more studies in other parts of the world will reveal the universality of such mechanisms in bird-dispersed species depending on nurse plants. The variation in favourable conditions for establishment under the canopy is most likely the selective pressure that caused the evolution of this type of germination response. Therefore such future studies should preferably be accompanied by studies that also record the spatial pattern of seed rain, seedling emergence and seedling survival under the canopy in combination with spatial variation in microclimate under the canopy.

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