The influence of light and temperature on the germination of two Ugandan medicinal trees

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Abstract

For reasons of the problems of establishment of some Ugandan trees in certain environments, we investigated the influence of temperature and light on germination and seedling growth of two locally threatened medicinal trees, *Hallea rubrostipulata* and *Sarcocephalus latifolius*, to facilitate their establishment. Field and controlled laboratory experiments were carried out to investigate the species germination requirements and seedling growth. Both species needed light to germinate. *Hallea rubrostipulata* had a temperature optimum of 25°C with 79% germination, while for *S. latifolius,* the total germination after 28 days was close to 60% at temperatures from 20 to 35°C. Seedlings of *S. latifolius* survived well at 35°C, while those of *H. rubrostipulata* died at this high temperature. Conversely, seedling of *S. latifolius* died at the low temperature of 15°C. However, in field experiment *S. latifolius* failed to germinate in the available degraded environments, probably because of predation and because the soil is not able to retain water long enough to support seedling growth. We, therefore, conclude that in this part of Uganda, nursery assistance is needed to establish healthy populations of *Sarcocephalus* and many other endangered trees.

Key words: conservation, germination, *Hallea rubrostipulata*, medicinal plants, *Sarcocephalus latifolius*, seedling growth

Résumé

En raison des problèmes que connaissent plusieurs arbres ougandais pour s'établir dans certains environnements, nous avons recherché l'influence de la température et de la lumière sur la germination et la croissance des plantules de deux arbres médicinaux localement menacés, *Hallea rubrostipulata* et *Sarcocephalus latifolius*, afin de faciliter leur établissement. Des expériences de terrain et d'autres contrôlées en laboratoire ont été réalisées afin de découvrir les exigences des espèces pour leur germination et la croissance des plantules. Les deux espèces ont besoin de lumière pour germer. La température optimale pour *H. rubrostipulata* était de 25°C avec 79% de germination, tandis que pour *S. latifolius*, la germination totale était près de 60% après 28 jours à des températures qui allaient de 20 à 35°C. Les jeunes plants de *S. latifolius* survivaient bien à 35°C alors que ceux de *H. rubrostipulata* mouraient à cette haute température. Par contre, les plants de *S. latifolius* mouraient à la basse température de 15°C. Pourtant, sur le terrain, *S. latifolius* n'a pas réussi à germer dans les environnements dégradés qui étaient disponibles, probablement à cause de la prédation et parce que le sol n'était pas à même de retenir l'eau assez longtemps pour permettre la croissance des jeunes plants. Nous en concluons donc que, dans cette partie de l'Ouganda, il faut une aide en pépinière pour établir des populations saines de *Sarcocephalus* et de nombreux autres arbres en danger.

Introduction

Most rural communites in developing countries rely on medicinal plants for their health care (Tabuti, Lye & Dhillion, 2003; Hamilton, 2004). Unfortunately, important medicinal trees are threatened by overexploitation and land use changes (Hamilton, 2004; Kala, Farooqueen & Dhar, 2004). In Uganda, for example, the forest cover has decreased from 13.7% to 3.6% of total land area during the last century (Arinaitwe, Pomeroy & Tushabe, 2000).

Important trees on which local livelihoods depend need to be conserved, which requires a good understanding of their seed and germination ecology (Oryem-Origa, 1999; Peters, 1999; Jäger & van Staden, 2000;

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Oryem-Origa, Kasenene & Magambo, 2004). However, such knowledge on indigenous trees in tropical Africa is very limited.

Many factors, including temperature and light, influence seed germination and seedling establishment. Optimum temperature for germination varies among species. Some have a wide range (Sparg, Kulcarni & van Staden, 2005), while others have a very narrow range (Yirdaw & Leinonen, 2002). The growth rate is the fastest in small seeded species (Fenner & Thompson, 2005; Warren & Adams, 2005; Cardillo & Bernal, 2006). Grime, Mason & Curtis (1981) found that most seeds weighing <0.1 mg were largely photoblastic. Most of positively photoblastic seeds react negatively to the low R : FR ratio of leaf filtered light (Fenner & Thompson, 2005).

In this study, we investigated the effect of temperature and light on germination and seedling growth of two locally important medicinal trees *Hallea rubrostipulata* (K. Schum.) J. F. Leroy (syn. *Mitragyna rubrostipulacea*) and *Sarcocephalus latifolius* (Sm.) E.A. Bruce, (syn. *Nauclea latifolia*) both of the family Rubiaceae. The bark of *H. rubrostipulata* was ranged as the most important treatment against malaria in a recent ethnomedicinal survey in Southern Uganda (Ssegawa & Kasenene, 2007). They found that there is a need to propagate *H. rubrostipulata* in nursery to conserve the natural population in the forest; but repeated germination experiments in two local nurseries at Malabigambo Forest have failed (Eilu, 2007).

Sarcocephalus latifolius is a shrub or small tree in seasonally moist soils of woodland savannas extending from Senegal to Uganda. In the beginning of this study, a focus group discussion with seven traditional healers from Gadumire ranked *S. latifolius* as one of the five most important medicinal plants, but getting difficult to find. The study of Tabuti (2007) confirms that it is overharvested and locally threatened.

Materials and methods

Material

Seeds of *H. rubrostipulata* were collected towards the end of May 2005 from one tree (0°56′60″S, 31°35′19″E; and altitude 1140 m). Each fruit is a capsule about 1-cm long containing numerous minute winged seeds. The species grows in a dense swamp forest in Sango Bay Forest Reserve, Rakai District close to Lake Victoria and near the border to Tanzania. The climax vegetation of the Reserve is evergreen oligotrophic rainforest, previously dominated by *Podocarpus*. The Reserve has a mean annual maximum temperature of 25–27.5°C, a mean annual rainfall of 1300–1500 mm, and there are 90–100 days of rain per year.

Sarcocephalus latifolius fruits were collected in March 2005 from two individual trees growing in disturbed savanna woodland in Kaliro District (1°02′23″N, 33°28′53″E; and altitude 1065 m). The fruit is an irregularly globose berry, 3–8 cm in diameter, containing thousands of minute seeds immersed in a pinkish flesh. Kaliro is situated in East Uganda south of Lake Kyoga. It has a mean annual maximum temperature of 30–32.5°C, mean annual rainfall of 1250–1300 mm, and there are 90–100 days of rain per year.

Seeds were collected from few trees because mature trees with ripe seeds are very rare. The *Hallea* seeds are also difficult to collect as they are 20 m up in the canopy, they easily fall to the ground as they ripen, and are impossible to find on ground because of small size.

Field growth experiment

In March 2005, a field germination experiment with *S. latifolius* was conducted in Gadumire in Kaliro District. Fruits of *S. latifolius* were soaked in water to separate the minute seeds from the dry fruits as recommended by Katende, Birnie & Tengnäs (1995) and then air dried in the shade. Seeds were sown in an agricultural fallow (>2 years) in an area dominated by disturbed wooded savannah. We tested for grazing, shade and different degrees of soil disturbance. The species failed to germinate and the experiment was repeated in November 2005 using the same plots but with different treatments. The experiments were performed in the long (March) and short (November) rainy seasons.

Germination experiment

The minute seeds were stored dry at room temperature for 3 (*H. rubrostipulata*) or 6 months (*S. latifolius*) before sowing. The germination study began in September 2005 and lasted 4 weeks. The average seed weights were 0.00712 mg for *H. rubrostipulata* and 0.0233 mg for *S. latifolius* (n = 50). The seeds were incubated in five controlled environment cabinets at constant temperatures of 15, 20, 25, 30 and 35°C under 12 : 12 h light/dark using Philip master (TDL 36W/830) with photosynthetic photon flux density (PPFD) at 400–700 nm values approximated 130 μmol m^{-2} s^{-1}.

Five pseudo replicates of 50 seeds of each species were germinated at each temperature in 9-cm Petri dishes on water saturated double filter paper. To prevent evaporation, the Petri dishes were placed in polyethylene bags. Every second day for 28 days, newly germinated seeds were counted and removed to another Petri dish. Germination was defined as radicle emergence. To determine the effect of continuous darkness, five pseudo replicates of each species were wrapped in aluminium foil and incubated in the same cabinets. The seeds under dark treatment were counted under safe green light. Distilled water was added regularly to the dishes as needed.

Seedling growth experiment

The study of seedling growth started in mid-January 2006 and lasted 12 weeks. Seeds were sown on saturated filter paper in 9-cm Petri dishes and placed in a controlled environment cabinet at 25°C. After germination, when the seedlings had unfolded the two cotyledons, the seedlings were transferred to pots (8 cm) using a standard potting compost mixed with perlite and placed in five growth chambers at constant temperatures of 15, 20, 25, 30 and 35°C under 12 : 12 h light/dark. Light sources were Osram Powerstar HQI-BT 400W, Osram, Munich, Germany supplemented by incandescent lamps to lower the R : FR ratio. PPFD values were 200 μmol m^{-2} s^{-1} (light to medium shade), R : FR 1.6 and relative humidity 90%.

Five seedlings of each species were harvested, dried and weighed at the time of planting (time 0). After week 4, four seedlings of *S. latifolius* and five of *H. rubrostipulata* plants from each temperature were harvested, separated into shoots and roots, dried at 70°C for 48 h and then weighed. Harvesting continued every second week until the twelfth week after planting.

Relative growth rate and statistical analysis

Relative growth rates (RGR) at the different temperatures and at different phases of seedling growth were determined by taking the natural logarithm of change in dry mass (W) of shoots or roots to change in time (t) as follows (Hunt *et al.*, 2002):

$$\mathrm{RGR} = \ln\left(\frac{W_2 - W_1}{t_2 - t_1}\right).$$

One-way ANOVA test for percentage germination versus temperature was run for both species. Two-way ANOVA was used to test if germination was significantly different between the two species. The Minitab statistical program was used for the tests.

Results

Germination

In the field experiments, no seeds of *S. latifolius* were observed to germinate. However, during the laboratory experiment, close to 60% of the same batch of seeds germinated at 20, 25, 30 and 35°C (Fig. 1). Germination was completed within 15 days at the temperatures 25 and 30° C. Total germination took more days (25) at 20 and 35 °C (Fig. 2). At 15 °C, no seeds germinated.

Hallea rubrostipulata had optimum germination (78.8%) at 25°C (Fig. 1). At 20 and 30 °C, germination were 59.6 and 67.2°C respectively. Most of the seeds had germinated within 6 days and germination was completed by the ninth day at 25 and 30°C (Fig. 2). No seeds of *H. rubrostipulata* germinated at 35°C. At the low temperature of 15°C, the capability of *H. rubrostipulata* to germinate was low (25%) and delayed.

For both species, very few seeds germinated in the dark (0.8% of *Hallea* at 20 and 25°C; 5.6% and 4% of *Sarcocephalus* at 20 and 25°C respectively).

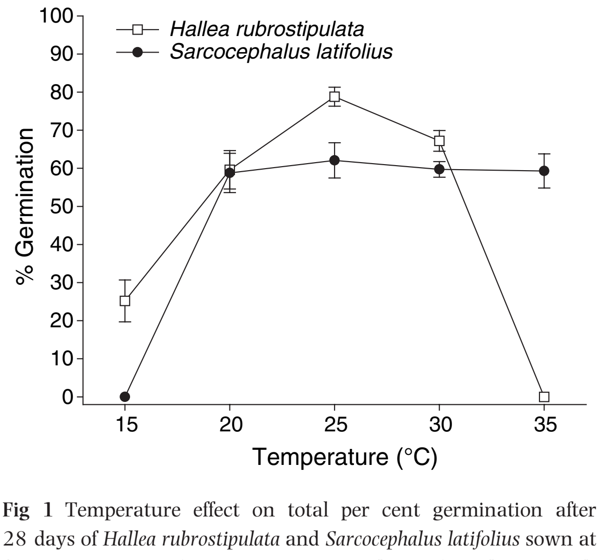

Fig 1 Temperature effect on total per cent germination after 28 days of *Hallea rubrostipulata* and *Sarcocephalus latifolius* sown at five temperatures (15, 20, 25, 30 and 35°C) and 12 : 12 h light/dark. Bars show standard errors of the mean (n = 5)

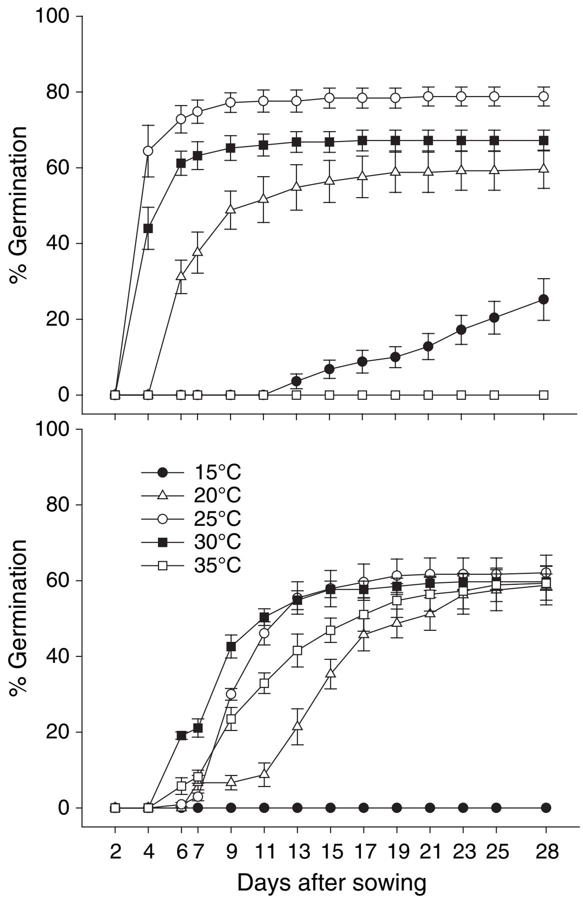

Fig 2 Cumulative per cent germination of *Hallea rubrostipulata* (above) and *Sarcocephalus latifolius* (below) sown in Petri dishes and incubated at five constant temperatures and 12 : 12 h light/dark for 28 days. Bars show standard errors of the mean (n = 5)

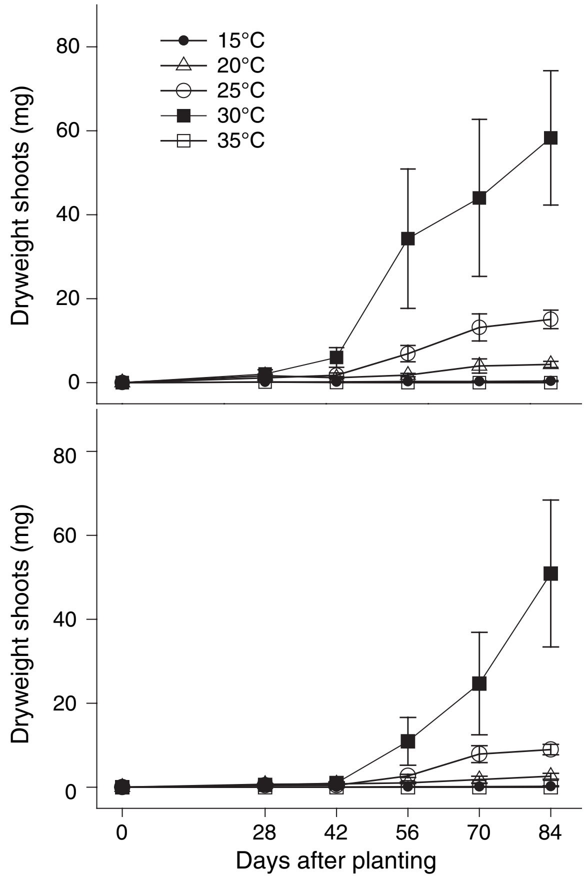

Fig 3 Dry weight of shoots and roots of *Hallea rubrostipulata* grown in pots at five constant temperatures and 12 : 12 h light/dark and harvested every second week from week 4 to week12 after planting (n = 5). Bars show standard errors of the mean

Seedling growth

Both species had the best growth at 30°C, and this was increasing linearly with time (Figs 3 and 4). For *S. latifolius*, the growth in terms of dry weight of the shoots was almost twice as high as the dry weight of roots, while for *H. rubrostipulata* dry weight of roots and shoots was almost similar. After 12 weeks, dry weight of *Sarcocephalus* roots and shoots were almost ten times higher than that of *Hallea. Sarcocephalus latifolius* had its second best growth at 35°C, while *Hallea* died at this high temperature.

In both species, RGR was the highest and showed the clearest trends in the first 4 weeks (Fig. 5, Table 1). *Sarcocephalus latifolius* showed a linear increase with temperature between the temperatures of 15 and 30°C in both root and shoot. *Hallea* had a somewhat lower and broader optimum of RGR between 25 and 30°C (Fig. 5). For the periods after 42 days, the RGR of *S. latifolius* declined, while for *H. rubrostipulata* it still increased for 14 days and then declined (Table 1).

After 9 months in greenhouse at the same environment, the two species had about the same height of 50 cm.

Two-way ANOVA for per cent germination versus species and temperature showed that there was effect of species on some of the temperatures ($F = 39.14$, $P < 0.001$).

Discussion

As *S. latifolius* seeds failed to germinate under disturbed conditions in wooded savanna, constraints like water stress and herbivory could have affected germination and

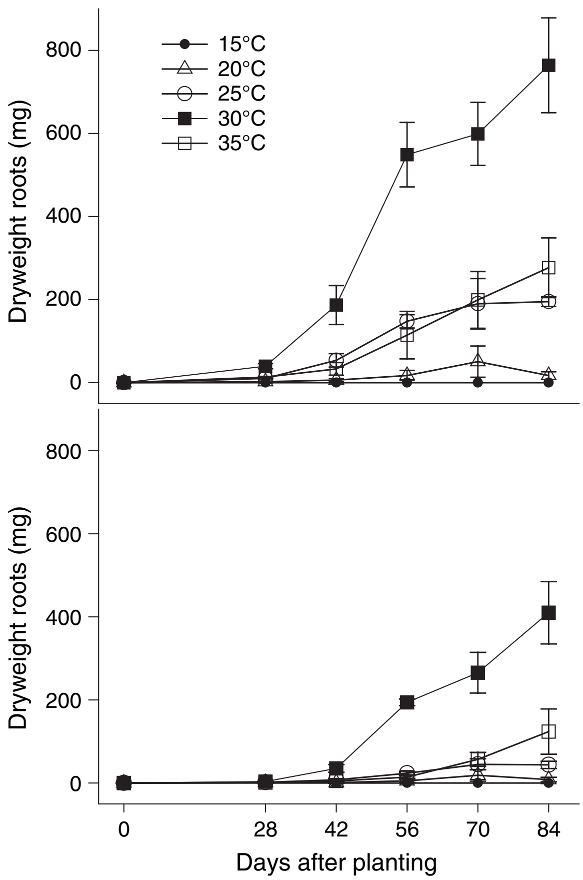

Fig 4 Dry weight of shoots and roots of *Sarcocephalus latifolius* grown in pots at five constant temperatures and 12 : 12 h light/dark and harvested every second week from week 4 to week 12 after planting (n = 4). Bars show standard errors of the mean

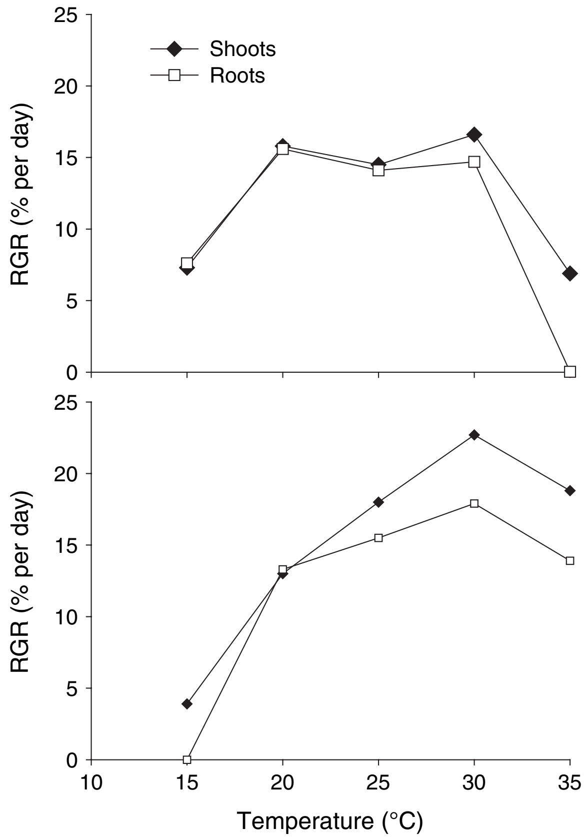

Fig 5 Relative growth rate (RGR) mg g^{-1} day^{-1} of *Hallea rubrostipulata* (above) and *Sarcocephalus latifolius* (below) during the first 28 days of the experiment

survival. As termites were abundant in and around the experimental field, they may have eaten or collected the small seeds of *S. latifolius*. The shaded plots were more protected against drought and sunburn, but lack of germination here could be because of low R : FR (Grime *et al.*, 1981; Yirdaw & Leinonen, 2002). This implies that the species cannot be established easily without nursery assistance.

As it is well known that species with seeds weighing <0.1 mg usually require light to germinate (Grime *et al.*, 1981; Fenner & Thompson, 2005), it was not unexpected that few seeds of *S. latifolius* and *H. rubrostipulata* germinated in darkness (0.8% of *Hallea* at 20 and 25°C; 5.6% and 4% of *Sarcocephalus* at 20 and 25°C respectively).

Although both species overlapped in terms of their preferred temperature for germination and early seedling establishment (20–30°C), *S. latifolius* had a higher temperature range than *H. rubrostipulata* and this reflected their natural ecological adaptations. The natural habitat where *S. latifolius* grows in Uganda has higher temperature ranges than those of Sango Bay Forest Reserve according to Atlas of Uganda (Anonymous 1967). *Hallea rubrostipulata* managed to establish at the lower temperature of 15°C, while *S. latifolius* failed. Simon *et al.* (1976) found that germination of tropical species declines dramatically at about 14°C and ceases at 10°C. This is consistent with our findings for *H. rubrostipulata,* but not with our findings for *S. latifolius* where the lower germination limit was between 15 and 20°C. The two tree species thus show an amazing adaptation of seed germination and early seedling growth to their prevailing environmental conditions.

As *Hallea* germinated after 4 days and many seeds were contaminated by fungi within 4 days, especially at 20°C, we conclude that rapid germination may be a way of

Table 1 Relative growth rate (RGR) of shoots and roots of *Hallea rubrostipulata* (*H*) and *Sarcocephalus latifolius* (*S*) grown at five temperatures and harvested five times from 28th to 84th day after planting

		RGR[b] (mg g^{-1} day^{-1})			
Treatment (days)[a]	Temp. (°C)	Shoots *H.*	Shoots *S.*	Roots *H.*	Roots *S.*
$T_2 - T_1$ (28 − 0)	35	69	188	25	139
	30	166	227	148	179
	25	145	180	141	155
	20	158	130	156	133
	15	74	39	8	−8
$T_3 - T_2$ (42 − 28)	35	−73	76	–[b]	118
	30	88	129	51	189
	25	35	136	1	116
	20	−29	72	11	21
	15	12	−169	−22	−92
$T_4 - T_3$ (56 − 42)	35	–[b]	103	–[b]	87
	30	145	90	197	142
	25	114	84	146	93
	20	35	86	21	116
	15	36	–[b]	53	–[b]
$T_5 - T_4$ (70 − 56)	35	–[b]	46	–[b]	115
	30	21	7	68	26
	25	54	21	89	54
	20	66	89	45	103
	15	2	–[b]	24	–[b]
$T_6 - T_5$ (84 − 70)	35	–[b]	27	–[b]	65
	30	23	20	60	36
	25	11	2	11	2
	20	7	90	30	−66
	15	29	–[b]	38	–[b]

[a]T is number of days from T_1: the day of potting.
[b]No data indicate that the plants have died.

escaping mortality by fungi, as suggested by Muhanguzi, Obua & Oryem-Origa (2002) for some Ugandan forest trees. The *Sarcocephalus* seeds germinated slightly later and were more resistant to fungal infection (about 50% of the seeds had germinated within 10 days at the same temperatures).

There were striking differences between the species in biomass allocation to shoots and roots. While at the end of the experiment, *Hallea* had almost as much biomass in roots as in shoots, *Sarcocephalus* had only about half as much biomass in the roots as in the shoots. The slow growth of the *Hallea* seedlings and the relatively large allocation to roots might be a strategy for adapting to an oligotrophic environment.

The high germination percentage (60% and 60–80%) after 3 or 6 months storage indicates that seeds of these species can be stored for considerable time, but the actual length of seed longevity must be studied further.

The light treatment given was suitable for seedling growth (200 μmol m^{-2} s^{-1}, R : FR = 1.6), which corresponds to light to medium-shade (Lee *et al.*, 1996, 1997; full sun: 1528 μmol m^{-2} s^{-1}, R : FR = 1.34; light shade: 400–600 μmol m^{-2} s^{-1}, understory shade: 13 μmol m^{-2} s^{-1}, R : FR = 0.20). Several studies have given the best seedling growth at moderate shade and high R : FR (Lee *et al.*, 1996; Yirdaw & Leinonen, 2002). Moderate shade results in better survival during the dry season (McLaren & McDonald, 2003; Lemenih, Gidyelew & Teketay, 2004).

To domesticate *Hallea* and *Sarcocephalus* successfully, it will be important to find or create habitats with the preferred light and temperature requirements of the species. Our field experiment indicates that natural establishment of *S. latifolius* in degraded environment is very difficult without nursery assistance. For *Hallea*, repeated germination experiments in the local nurseries at Malabigambo Forest also failed.

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