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Short communication

Germination response and viability of an endangered tropical conifer *Widdringtonia whytei* seeds to temperature and light

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Abstract

The tropical conifer *Widdringtonia whytei* Rendle is an endangered species endemic to Mulanje Mountain in Malawi. A study was conducted for the first time under controlled conditions in order to assess the effects of temperature and light on germination and viability of *W. whytei* seeds. Seeds incubated at a constant temperature of 20 °C attained the highest cumulative germination percentage (100%) followed by 87% germination under fluctuating temperatures of 15 °C night/25 °C day. No seed germination occurred at temperatures below 15 °C. Seeds that failed to germinate at temperatures below 15 °C showed the highest (>90%) viability compared to the seeds incubated at 25 °C (60%). Across temperature regimes, germination was significantly higher under light (44.7%) than dark (35.6%) conditions. It is concluded that temperature is one of the critical factors for germination of *W. whytei* seed. The ability of *W. whytei* seeds to germinate both in light and darkness implies that the species would unlikely form a persistent soil seed bank, an attribute which is common in species that survive in habitats frequently disturbed by fires.
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1. Introduction

The tropical conifer *Widdringtonia whytei* Rendle represents one of nine Southern African conifer species (Mustart, 2008). It is endemic to Mulanje Mountain in Malawi (Bayliss et al., 2007). Further, *W. whytei* is an endangered species that has been given global importance due to being listed by the IUCN onto a list of 43 conifer species worthy of special conservation attention (Bayliss et al., 2007). The species has undergone a drastic decline due to excessive exploitation and the effects of very intense wildfires (Bayliss et al., 2007; Chapman, 1995). Consequently, *W. whytei* populations are highly fragmented on the Mountain and restricted to ravines and hollows on the plateaus, below cliffs and in the gorges where the terrain affords some protection from fires and logging, to some extent. Current *W. whytei* fragments show very poor natural regeneration (Makungwa, 2004). Until recently, efforts towards understanding its ecology and conservation have been very limited. To date, no information has been presented on the ecological requirements of this species including the germination of its seeds.

Germination is one of the most critical stages in the life cycle of any plant (Thompson and Ooi, 2010), and germination requirements are often assumed to be adaptations to the particular habitats where the species occur (Meyer et al., 1990). Each species has particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Simons and Johnston, 2006). Therefore, understanding these requirements is very important in the conservation of endangered species such as *W. whytei*. Temperature and light are important ecological factors that regulate seed germination of

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many plant species (Baskin and Baskin, 1998; Botha et al., 1982; Jarvis and Moore, 2008). Both low and high temperatures have been reported to inhibit seed germination in several species (Amri, 2010; Teketay, 1994).

The role of light in the germination of seeds has been studied by several authors (Baskin and Baskin, 1998; Botha et al., 1982). Some seeds germinate equally well in light and darkness (Baskin and Baskin, 1998), while others germinate more readily either only under light (Baskin and Baskin, 1990; Colbach et al., 2002; Grime et al., 1981) or darkness (Baskin and Baskin, 1990; Thanos et al., 1989). In addition, light requirements for seed germination may vary with changes in temperature (Baskin and Baskin, 1998; Farmer et al., 1984; McLemore and Hansbrough, 1970). Although many studies have been conducted on the effects of temperature and light on seed germination, temperature and light germination requirements are relatively unexplored in many plant species (Cony and Trione, 1996).

No studies exist on the effects of temperature and light on seed germination of *W. whytei*. Therefore, the objective of this study was to determine the temperature and light requirements for germination of its seeds.

2. Material and methods

2.1. Site description

Mature cones of *W. whytei* were collected and bulked in August 2009 from a large population (86 trees of different sizes) at Sombani (15°52′41″S and 35°40′89″E) on Mulanje Mountain. The temperatures on Sombani vary from 5 °C in winter to about 28 °C in summer. The lowest temperatures usually occur in June/July while the warmest temperatures occur in October. More than 2200 mm of rainfall is received on the site annually. The altitude at Sombani ranges from 1660 to 2265 m above sea level (Lawrence et al., 1994). Seeds were obtained after the cones were placed and allowed to open in the shade at the Forestry Research Institute of Malawi, Zomba. In the drying shade temperature varied between 22 and 30 °C and the relative humidity ranged between 30% and 38%. Seeds were dried to 5% moisture content (Gondwe, 2008). The seed moisture content (MC) was determined gravimetrically and expressed on a fresh weight basis. The dry mass was measured after heating seeds in an oven for 17 h at 103 °C according to the International Seed Testing Association rules (ISTA, 2003). Seeds of *W. whytei* are 25 mm long and 12 mm wide with a wing and are dark in colour (Pauw and Linder, 1997).

2.2. Seed germination and viability

Germination tests included seven temperature regimes with light and dark treatments. Each treatment was replicated five times with 20 seeds per replicate. Seeds were placed in 9-cm diameter Petri dishes filled with Agar-agar, and incubated in LTGC-40 growth cabinets at constant temperatures of 5, 10, 15, 20 and 25 °C and two alternating temperatures of 15 °C night and 25 °C day and 10 °C night and 20 °C day temperatures. These alternating temperatures will be referred to as 15N/25D and 10N/20D in the following discussion. Seeds under the light and dark treatments were exposed to a photoperiod of 12 h light/12 h dark hours or continuous dark respectively. Light was provided by the cool-white 40-W fluorescent bulbs with 1000 lx (ISTA, 2003). Fluorescent tubes were used because they emit considerable red but little far-red light (Baskin and Baskin, 1998). Dark condition was provided by wrapping Petri dishes in three layers of aluminum foil (Baskin and Baskin, 1998). Germination counts under light treatments were recorded daily for 30 days while samples in darkness were opened on the final day of the experiment. A seed was considered to have germinated when the radicle was observed.

Complete germination rarely occurs when undertaking seed germination experiments (Scott et al., 1984). Seeds that failed to germinate at the end of the observation period were tested to see if they were viable. Vital staining with 2,3,5-triphenyl-2*H*-tetrazolium chloride (tetrazolium test) was used in this study and was followed by cutting tests for confirmation. Seeds cleaned with water and soaked in 1% tetrazolium chloride solution for 8 h at 25 °C (ISTA, 2003). Pink embryos were scored as live and total count was expressed as percentage of viable seeds. The tetrazolium test (Baskin and Baskin, 1998) is a rapid biochemical test of seed viability compared to the more slow germination assays (Donald, 1994). The tetrazolium test becomes a dormancy test when it is done on the ungerminated seeds left after a standard germination test or as a separate test alongside the germination test.

2.3. Statistical analysis

Final germination percentages were calculated for each treatment as the cumulative number of germinated seeds with normal radicles out of the total of 20 seeds per replicate. Since the germination percentage data violated the normality assumptions (Shapiro–Wilk and Kolmogorov–Smirnov tests), arcsine transformation was applied before subjecting the data to analysis of variance (ANOVA) using the GLM procedure of the SAS software version 9.1 (SAS Institute Inc., USA). Means were separated using Tukey's studentized range test (HSD).

3. Results

3.1. Germination response

Cumulative germination percentage after 30 days significantly differed with temperature ($F=211.1$, $P<0.001$, $df=6$), light condition ($F=26.8$, $P<0.001$, $df=1$) and their interaction effect ($F=5.8$, $P<0.001$, $df=6$). The highest cumulative germination percentage (100%) was recorded in seeds incubated at a constant temperature of 20 °C, followed by alternating temperatures of 15N/25D, which achieved 87% germination (Fig. 1). The lowest germination percentage (0%) was recorded at constant temperatures of 5 and 10 °C. Across temperature regimes, germination was significantly higher under light than dark conditions (Fig. 2a). The highest germination was observed at 20 °C under both light and dark conditions. However, germination in the dark was slightly higher than in the light under alternating

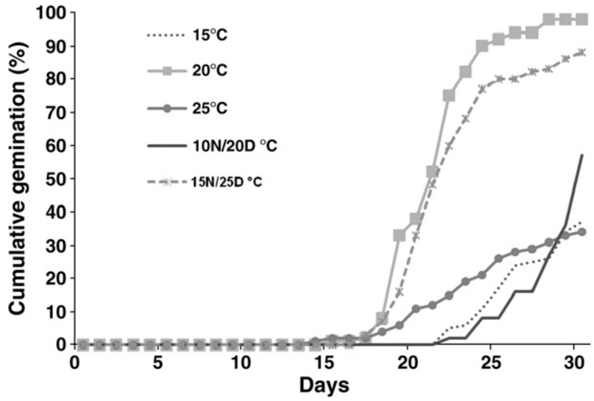

Fig. 1. Cumulative distribution of germination of *Widdringtonia whytei* seeds over the germination period.

temperatures of 10N/20D. The cumulative distribution shows that germination reaches 100% within 25 days at a constant temperature of 20 °C while at the alternating temperatures of 15 N/25D it took almost 30 days to attain 80% germination (Fig. 1). It took about 16 days for seeds to start germinating at constant temperature of 20 °C and alternating temperatures of 15 N/25D. It took 21 days for seeds to start germinating at constant temperature of 15 °C and alternating temperature 10 N/20D (Fig. 1). Germination remained below 50% either at constant temperatures below or above 20 °C.

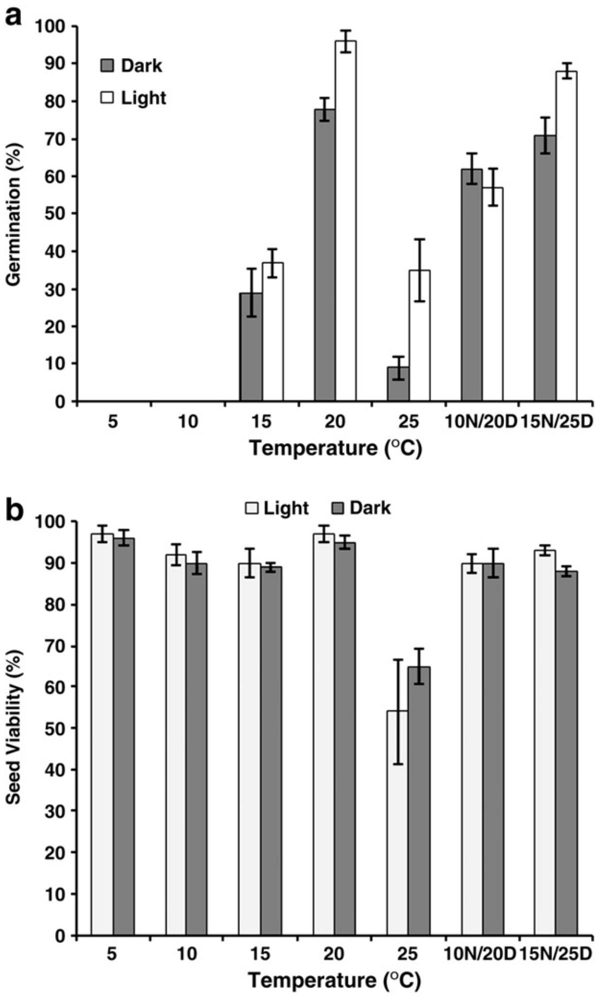

Fig. 2. The effect of different temperature and light conditions on (a) mean germination and (b) viability of *Widdringtonia whytei* seeds after 30 days. Bars represent mean ± standard error.

3.2. Seed viability

After 30 days of germination assay, seeds that failed to germinate at constant temperatures of 5 and 10 °C both under light and dark conditions showed >90% viability (Fig. 2b). This was followed by seeds that were germinated at 15 °C constant temperature under both light and dark conditions. The lowest overall seed viability was recorded on seeds incubated at 25 °C (68%). Many ungerminated seeds under dark conditions remained viable across temperatures regimes (Fig. 2b).

4. Discussion and conclusions

W. whytei seed germination exhibited a parabolic relation with the optimal temperature being about 20 °C. This agrees well with the mean annual temperature distributional range on Mulanje Mountain. Seeds germinated faster at temperatures between 15 and 25 °C. This behavior is similar to the germination requirements observed in other tropical montane tree species (Xiao et al., 2010). In the field, this temperature range occurs in early December during the rainy season. This suggests that maximum germination potential of *W. whytei* seeds would occur around December. *W. whytei* seed germination was higher at constant than at alternating temperatures. Although seed germination was higher at constant temperatures, alternating temperatures have been found to be more favourable for germination than constant ones (Thompson and Grime, 1983), since seeds are exposed to alternating and not constant temperatures in natural habitats (Baskin and Baskin, 1998).

The results from viability tests revealed that *W. whytei* seeds which did not germinate at low temperatures of 5 or 10 °C were mostly viable. This is in agreement with earlier reports that germination of most tropical species ceases at 10 °C (Khan and Ungar, 1999; Simon et al., 1976). Low temperatures (<15 °C) experienced on the forest floors of Mulanje Mountain during winter (June and July) (Chapman, 1995), could probably restrict seed germination by inducing dormancy. Lack of germination when seeds are exposed to low temperatures also suggests that little or no winter germination would take place in the field, which would consequently reduce the risk of higher seedling mortality later during the dry season. Furthermore, seeds dispersed in winter would partly contribute to the soil seed bank which is dominated by seeds dispersed in the dry season (August to October) (Chanyenga et al., 2011). In contrast, high temperature of 25 °C increased seed mortality suggesting that temperatures higher than 25 °C may prevent germination of *W. whytei* as a result of increased seed mortality.

Although seeds germinated in both light and darkness, more seeds germinated under light than dark conditions. Similar responses were reported for other species (Grime et al., 1981). The ability of seeds to germinate under light and dark conditions means that *W. whytei* seeds can germinate either buried or exposed on the soil surface disputing earlier suggestions that *W. whytei* seed requires light to germinate (Chapman, 1995). These characteristics suggest that *W. whytei* cannot form a persistent soil seed bank (Fenner and Thompson, 2005; Pons, 2000). From this study, it can be concluded that temperature is one of the critical factors for germination of *W. whytei* seed and that the seed does not require light to germinate.

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References

Amri, E., 2010. Germination of *Terminalia sericea* Buch ex DC. seeds. Effects of temperature regime, photoperiod, gibberellic acid and potassium nitrate. American-Eurasian Journal of Agriculture and Environmental Science 8, 722–727.

Baskin, J.M., Baskin, C.C., 1990. The role of light and alternating temperatures on germination of *Polygonum aviculare* seeds exhumed on various dates. Weed Research 30, 397–402.

Baskin, C.C., Baskin, J.J., 1998. Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, California, p. 666.

Bayliss, J., Makungwa, S., Hecht, J., Nangoma, D., Bruessow, C., 2007. Saving the island in the sky: the plight of the Mount Mulanje cedar *Widdringtonia whytei* in Malawi. Oryx 41, 64–69.

Botha, F.C., Grobbelaar, N., Small, J.G.C., 1982. Seed germination in *Citrullus lanatus*. 1. Effect of white light and growth substances on germination. South African Journal of Botany 1, 10–13.

Chanyenga, T.F., Geldenhuys, C.J., Harvey, J., 2011. Variation in seed rain from *Widdringtonia whytei* growing in different conditions on Mulanje Mountain in Malawi. Southern Forests: a Journal of Forest Science 73, 123–129.

Chapman, J.D., 1995. The Mulanje Cedar: Malawi's National Tree. Montfort Press, Limbe, Malawi.

Colbach, N., Chauvel, B., Durr, C., Richard, G., 2002. Effect of environmental conditions on *Alopecurus myosuroides* germination. I. Effect of temperature and light. Weed Research 42, 210–221.

Cony, M.A., Trione, S.O., 1996. Germination with respect to temperature of two Argentinean *Prospis* species. Journal of Arid Environments 33, 225–236.

Donald, W.W., 1994. A method of validating the tetrazolium assay for testing viability of dormant jointed goatgrass (*Aegilops cylindrica*) seed. Weed Science 42, 502–508.

Farmer, R.E., Charrette, P., Searle, I.E., Tarjan, D.P., 1984. Interaction of light, temperature, and chilling in the germination of black spruce. Canadian Journal of Forest Research 14, 131–133.

Fenner, M., Thompson, K., 2005. The Ecology of Seeds. University Press, Cambridge, pp. 110–135.

Gondwe, D., 2008. Influence of cone maturity on the quality of *Widdringtonia whytei* seed. MSc Thesis, University of Malawi.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., Mowforth, M.A.G., Neal, A.M., Shaw, S., 1981. A comparative study of germination characteristics in a local flora. Journal of Ecology 69, 1017–1059.

ISTA, 2003. International Rules for Seed Testing. ISTA, Bassersdorf, Switzerland.

Jarvis, J.C., Moore, K.A., 2008. Influence of environmental factors on *Vallisneria americana* seed germination. Aquatic Botany 88, 283–294.

Khan, M.A., Ungar, I.A., 1999. Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. Great Basin Naturalist 59, 144–145.

Lawrence, M., Oshino, A., Chinguwo, C., 1994. Mulanje cedar (*Widdringtonia cuppressoides* Endlicher) inventory. FRIM Report 94010 ODC 524.61.174.

Makungwa, S., 2004. Inventory results of Mulanje cedar resources on Mulanje Mountain. A report submitted to Mulanje Mountain Conservation Trust.

McLemore, B.F., Hansbrough, T., 1970. Influence of light on germination of *Pinus palustris* seeds. Physiology of Plant 23, 1–10.

Meyer, S.E., Monsen, S.B., McArthur, E.D., 1990. Germination response of *Artemisia tridentata* (Asteraceae) to light and chill, patterns of between-population variation. Botanical Gazette 151, 176–183.

Mustart, P.J., 2008. A synthesis of information on *Widdringtonia cedarbergensis* (The Clanwilliam cedar). Report of the Botanical Society of South Africa. (12p.).

Pauw, C.A., Linder, H.P., 1997. Tropical African cedars (*Widdringtonia*, Cupressaceae): systematic, ecology and conservation status. Botanical Journal of the Linnean Society 123, 297–319.

Pons, T.L., 2000. Seed responses to light, In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities, Second Edition. CABI Publishing, Wallingford, UK, pp. 237–260.

Scott, S.J., Jones, R.A., Williams, W.A., 1984. Review of data analysis methods for seed germination. Crop Science 24, 1192–1199.

Simon, E.W., Minchin, A., McMenamin, M.M., Smith, J.M., 1976. The low temperature limit for seed germination. New Phytologist 77, 301–311.

Simons, A.M., Johnston, M.O., 2006. Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet hedging. Evolution 60, 2280–2292.

Teketay, D., 1994. Germination ecology of two endemic multipurpose species of *Erythrina* from Ethiopia. Forest Ecology and Management 65, 81–87.

Thanos, C.A., Georghiou, K., Skarou, F., 1989. *Glaucium flavum* seed germination. An ecophysiological approach. Annals of Botany 63, 121–130.

Thompson, K., Grime, J.P., 1983. A comparative study of germination responses to diurnally-fluctuations temperatures. Journal of Applied Ecology 20, 141–156.

Thompson, K., Ooi, M.K.J., 2010. To germinate or not to germinate: more than just a question of dormancy. Seed Science Research 20, 209–211.

Xiao, C., Wang, X., Xia, J., Liu, G., 2010. The effect of temperature, water level and burial depth on seed germination of *Myriophyllum spicatum* and *Potamogeton malaianus*. Aquatic Botany 92, 28–32.

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