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Full Length Research Paper

Germination response of threatened African medicinal barberry (*Berberis holstii*) under light, stratification and temperature treatments

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Berberis holstii **is the only African representative of the Palearctic genus, Berberis. In Malawi, it is only found on Nyika Plateau where it is intensively and illegally harvested despite legislation banning its collection. Efforts to propagate it by seed have proved futile. This study investigated its germination response under light and stratification treatments. Ripe seeds were germinated under light/dark and chilling conditions, and constant/alternate temperature regimes. Seeds germinated equally well in light and dark conditions except under 20/5°C temperature where stratified seeds exposed to light failed to germinate. Under constant temperature, seeds germinated slightly better in light (about 50%). Seeds that were in dark germinated better under alternating temperatures (up to 80%). Because germination is not restricted by light, the species is unlikely to form a soil seed bank.**

Key words: Barberry, photoblastism, temperature, stratification.

INTRODUCTION

Berberidaceae family

The Berberidaceae family, also known as barberry, comprises 17 genera and ~ 650 species most of which are distributed in northern temperate areas and sub-tropical mountains (Junsheng et al., 2006; Watson and Dallwitz, 1992). The genus Berberis comprises about 500 species which are indigenous to Europe, Africa, Asia and North and South America (Junsheng et al., 2006). *Berberis holstii* Engl. is the only species endemic to Africa. *B. holtii* is used for medicinal purposes, hedges and firewood (Kokwaro, 1993; Bekele-Tesemma et al., 1993; Hedberg et al., 1982). In Malawi, it is regarded as an important traditional resource because of its reputed medicinal value (Burrows and Willis, 2005). However, the plant is only found on Nyika plateau in Malawi's Nyika National Park where it is intensively and illegally harvested despite legislation banning harvesting of the plant. Leaves are the only parts that are used and this involves uprooting entire plants. Efforts to propagate it by seed outside the park have been unsuccessful and there are concerns that the plant might be threatened. Despite the concerns, little is known about *B. holstii.* The study was therefore undertaken to investigate seed germination requirements and seed viability to generate information necessary for *ex-situ* propagation.

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MATERIALS AND METHODS

Study species

B. holstii, also known as Holst's barberry is an Afromontane endemic. It is distributed in seven countries namely Ethiopia, Somalia, Kenya, Uganda, Tanzania, Zambia and Malawi (Polhill, 1966; Hedberg et al., 1982). Malawi is the southern most limit of this range and it is the only locality known in the Flora Zambesiaca region (Wild, 1960; Dowsett-Lemaire, 1985; Burrows and Willis, 2005).

Locally known as Kayunga in Malawi, *B. holstii* is an important plant species in Malawi due to its medicinal uses. Some of the uses include coughs, malaria, stomachache, sexually transmitted infections, pneumonia, asthma, backache, hematuria, menorrhagia, body pains and sore throat (Maliwichi-Nyirenda et al., 2011). *B. holstii* is a woody shrub which grows in open upland woodland, edges and glades of upland rain-forest, upland evergreen bushland and *Juniperus-Hagenia-Olea* forest (Polhill, 1966; Bekele-Tesemma et al., 1993). Given the right conditions and sufficient time, the plant grows up to 5 to 6 m. It has 1 to 4 cm long tripartite spines, short axillary shoots and long oval berries, which turn from green to dark purple when ripe. Each fruit has 1 to 4 seeds (White et al., 2001; Polhill, 1966; Bekele-Tesemma et al., 1993). *B. holstii* regenerates through seeds, it flowers in October/November and fruits ripe in May/June.

Study area

The study took place on Nyika Plateau within Nyika National Park, northern Malawi. It is located between 10°15'S to 10°50'S longitude, 33°35'E to 34°05'E latitude, and 600 to 2607 m altitude (Brass, 1954; Dowsett-Lemaire, 1985; Department of National Parks and Wildlife, 2004). The park, shared between Malawi and Zambia, comprises the plateau, hills and escarpments (Department of National Parks and Wildlife, 2004). Nyika is the wealthiest habitat in as far as biological resources are concerned (Estes, 2001) and it is one of Africa's Centres of Plant Diversity (Kurzweil, 2000). It sustains rare, endemic and endangered species (Lemon, 1968) and is tentatively listed as a world heritage centre. *B. holstii* is restricted to the plateau, located between 1800 and 2607 m altitude (Burrows and Willis, 2005). It has distinct climate of mists and occasional frosts. It is dominated by grasslands and relict patches of montane evergreen forests; an indicator of persistent fires (Thatcher, 1974).

Seed collection and storage

Mature fruits (ripe and dark purple in colour) were collected in May/June. The flesh was removed and the seeds were washed, dried and stored in paper bags. In the laboratory, half the seeds collected were packed in plastic bags, labelled and stored in a refrigerator at 5°C (stratification treatment). The other half was stored at room temperature. The seeds were kept in this state until germination trials were undertaken.

Prior to the trials, the seeds were sterilised by submerging them in 10% Sodium hypochlorite for 5 min. They were later rinsed three times in distilled water and drained (Fuller and Pizzey, 2001; Rodriguez-Ortega et al., 2006).

Seed sowing

0.8% water agar was prepared, autoclaved at 120°C for 15 minutes, and poured into Petri dishes under a laminar flow hood (Fuller and Fuller, 1995). After the agar was set, the seeds were evenly distributed onto it using sterilised pincers. The sown seeds were exposed to light, stratification and temperature treatments with each treatment comprising 100 seeds in five samples (Petri dishes) of twenty seeds each.

Germination treatments

Light/dark

The response of seeds to light (photoblastism) was investigated in 9 cm diameter Petri dishes in a Sanyo growth cabinet at a constant temperature of 24°C with a 12:12 h day-light photoperiod. Seeds under the light treatment were exposed to this daily photoperiod. Those in darkness were wrapped in three layers of aluminium foil and kept in the same cabinet (Baskin and Baskin, 1998; Conner, 1987).

Germination under the light treatment was recorded every 24 h, while the samples in darkness were opened on the final day of the experiment (Baskin and Baskin, 1998).

Temperature

Seeds were germinated under constant temperature of 24°C and fluctuating temperatures of either 20/10 or 20/5°C. The latter two regimes roughly simulated the two extreme seasonal conditions that occur on the Nyika Plateau: the warm, rainy season and the cold winter. These experiments were also replicated for stratified and non-stratified (control) seeds, as well as for light/dark conditions, as in the constant temperature experiment described in the foregoing. Each treatment comprised five Petri dishes containing twenty seeds each.

Data analysis

Curve-fitting of the cumulative germination curves (the proportion of germinated seeds through time) was employed to compare treatment responses (Scott et al., 1984). A *t*-test was used to determine differences between treatments (Zar, 1999).

RESULTS

Germination response

Constant temperature

Under constant temperature, chilled and non-chilled seeds germinated equally well in light. The course of germination was as indicated subsequently (Figures 1a and b). The onset of germination occurred rather quickly: 3 days for chilled seeds and 4 days for unchilled ones. Under dark conditions, germination was higher for seeds that were pre-stored at room temperature than pre-chilled seeds (58 and 46%, respectively). There was no significant difference in the final percentage of germination (chilled = 72%; non-chilled = 68%) ($t = 0.539$; df = 18; $p>0.05$).

Fluctuating temperature

Germination under fluctuating temperature of 20/10°C was higher than at 20/5°C (Figures 2 and 3) especially when the seeds were pre-stored at room temperature (Figure 2a). Unlike under constant temperature where germination in light was higher than germination in dark (Figure 1), germination was higher for seeds that were germinated in dark than those germinated in light (Figures 2a, 3a and 3b). However, for pre-chilled seeds that were germinated at 20/10°C, germination in light was higher than germination in dark (Figure 2b). Pre-chilled

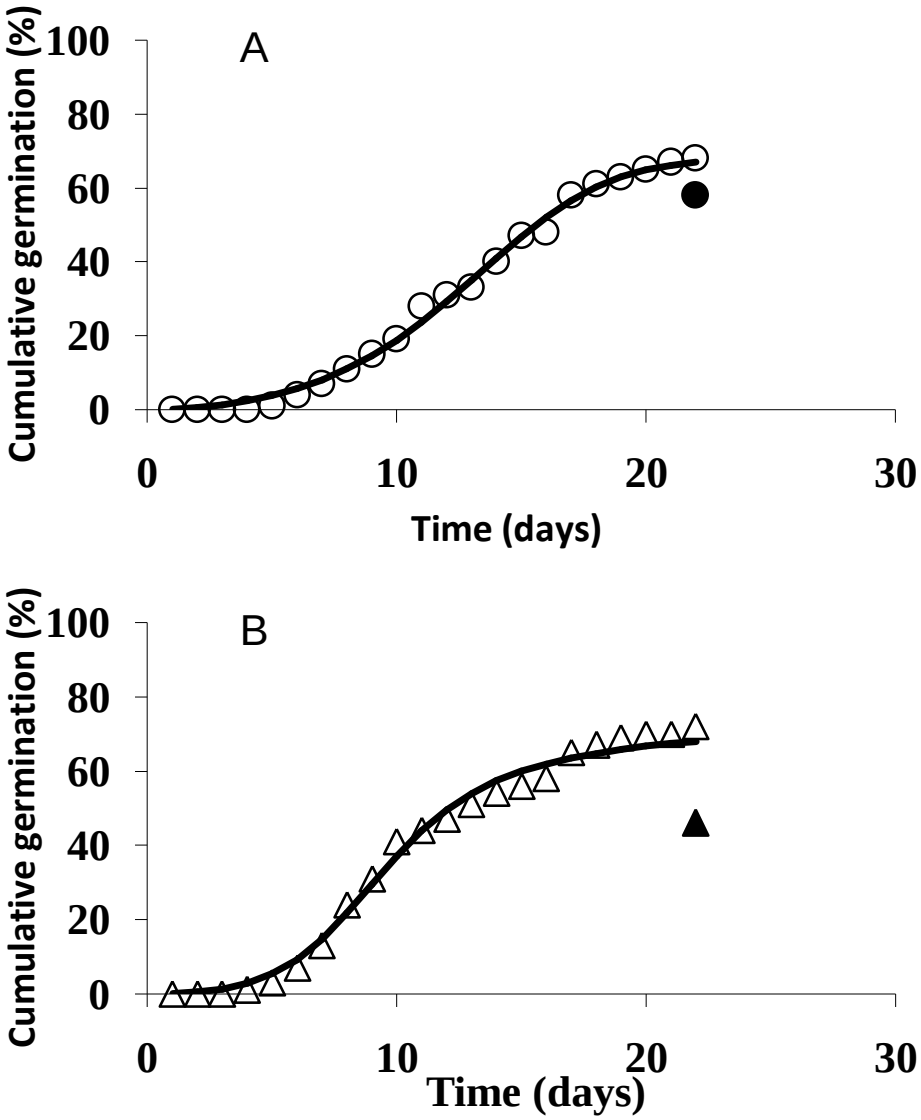

Figure 1. Germination response of *Berberis holstii* seeds under constant temperature of 24°C (a). Germination was done in light (open symbol) and dark conditions (filled symbol). The seeds were either pre-stored at room temperature (a) or pre-chilled (b).

seeds that were germinated in light at 20/5°C did not germinate at all (Figure 3b).

Photoblastism

Germination was not dependent on light. The seeds germinated in light and dark. Nonetheless, they germinated better in light than in dark under the constant temperature of 24°C (Figure 1), and the difference between light and dark treatments was significant (t = 2.924, df = 18, p<0.05).

The seeds germinated better at a constant temperature of 24°C and at the fluctuating 20/10°C temperatures in the dark (Figures 1 and 2). Under these conditions they exhibited ~70 to 80% germination. Germination at 20/5°C in the dark was 23% (Figure 3).

DISCUSSION

Although seeds did not require stratification to germinate, cold-stratified seeds germinated faster than those kept at room temperature. This is typical of many temperate species; they require cold stratification to germinate (Fountain and Outred, 1991). This is also expected for *B. holstii* as it inhabits climatically temperate Nyika plateau (Meadows, 1982).

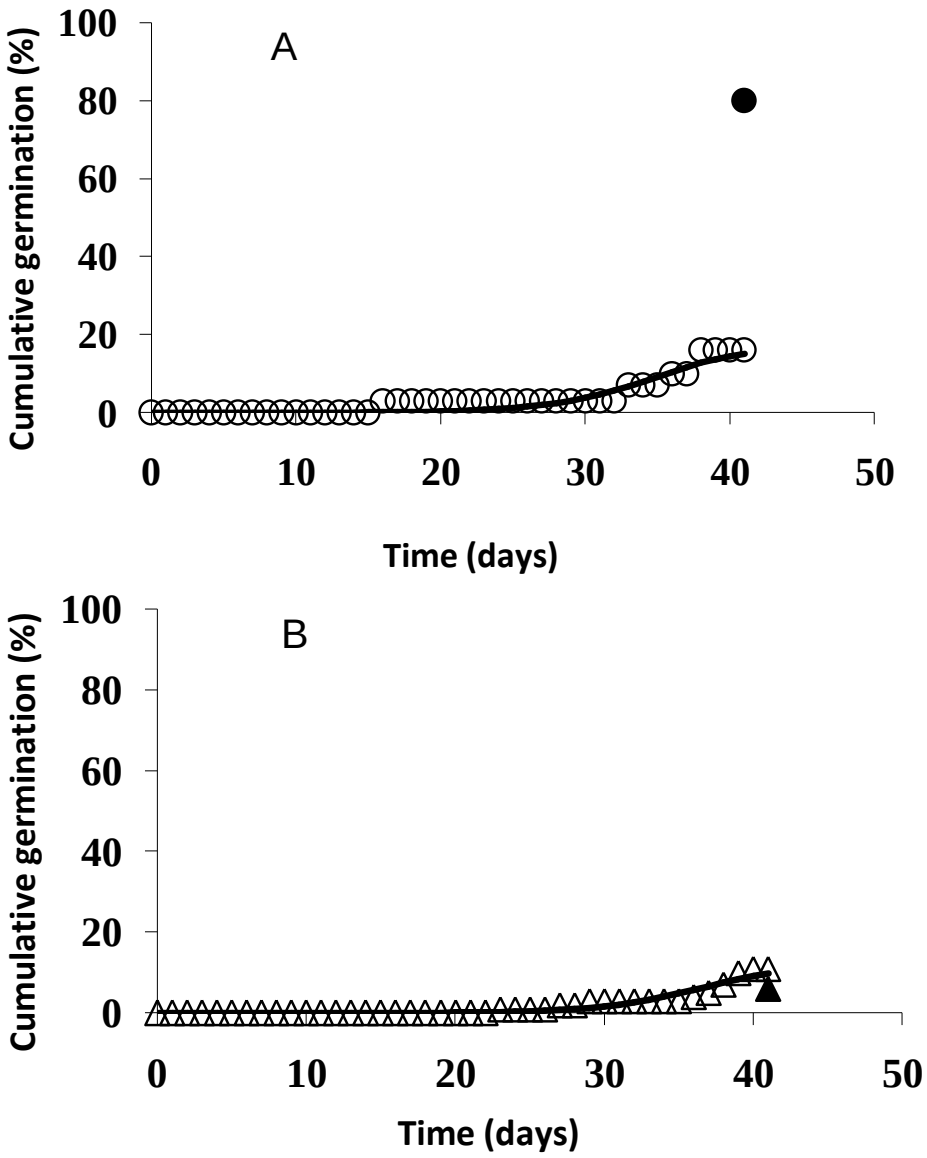

Figure 2. Germination response of *Berberis holstii* seeds under fluctuating temperature of 20/ 10°C. Germination was done in light (open symbol) and dark conditions (filled symbol). The seeds were either pre-stored at room temperature (a) or pre-chilled (b).

While seeds germinated in both light and darkness under a constant temperature of 24°C, the final percentage of germination was lower in the dark. Most of the seeds that did not germinate were as healthy as the ones that germinated except the few that were attacked by pathogens. The higher germination in the dark than under light at a fluctuating temperature of 20/10°C suggests that the aluminium foil may have buffered the changes in temperature in the growth cabinet.

Ecologically, the ability of the seeds to germinate in light and dark means that the seeds can germinate either buried or exposed on the surface of the soil, provided other factors, such as adequate temperatures necessary for germination, is also present. These characteristics suggest that *B. holstii* cannot form a persistent soil seed bank (Fenner and Thompson, 2005; Pons, 2000).

Implications on dormancy

Based on the trials conducted in this study, it is evident that chilling and light play a role in breaking dormancy. When combined with light, chilling had the effect of breaking dormancy under constant temperature. Light also broke dormancy under constant temperature, but it inhibited germination under alternate temperatures. The

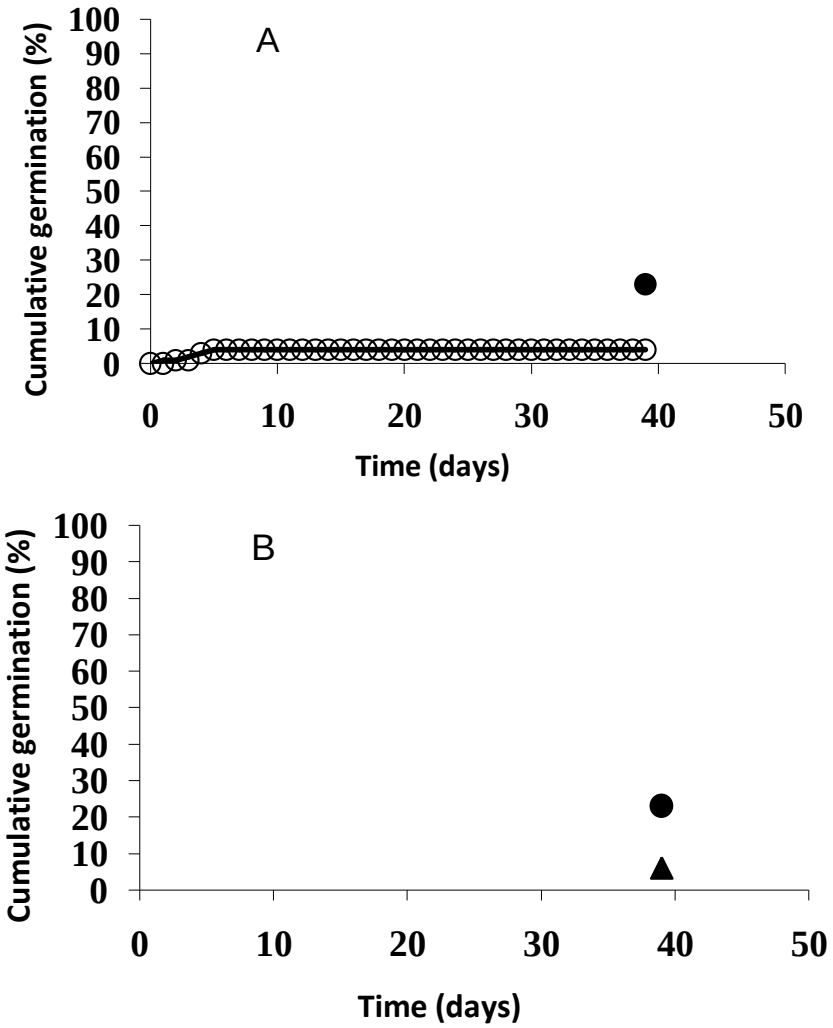

Figure 3. Germination response of *Berberis holstii* seeds under fluctuating temperature of 20/5°C. Germination was done in light (open symbol) and dark conditions (filled symbol). The seeds were either pre-stored at room temperature (a) or pre-chilled (b). For pre-chilled.

most effective temperature to break dormancy was 20°C; however, fluctuating temperatures, particularly 20/5°C had a negative effect on germination.

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