Effects of temperature and light on germination and early seedling development of the pine pink orchid (*Bletia purpurea*)

TIMOTHY R. JOHNSON and MICHAEL E. KANE
Plant Restoration, Conservation and Propagation Biotechnology Laboratory, University of Florida, Department of Environmental Horticulture, Gainesville, Florida 32611-0675, USA

Abstract

Orchid seed physiology is a poorly understood phenomenon owing to an emphasis on production and the challenges associated with propagating orchids from minute seed. We investigated the role of simulated south Florida temperatures and illumination (dark and 12 h photoperiod) in regulating germination and seedling development using asymbiotic seed germination assays of *Bletia purpurea*. Our objectives were to determine whether *in situ* germination is limited by seasonal temperatures and to determine whether temperature alters responses to illumination. *Bletia purpurea* seeds were able to germinate to >90% under all treatments. The greatest germination after 3 weeks was observed at 29/19°C under continual darkness and at 25°C under dark and illuminated conditions. The slowest germination was observed at simulated winter temperatures (22/11°C). Illumination initially inhibited germination and development, but resulted in equal or greater development by week six. Germination under 22/11°C was strongly inhibited by illumination, indicating an interaction between temperature and light sensing systems.

Keywords: abiotic effects, asymbiotic germination, illumination, Orchidaceae, seed.

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Introduction

The importance of temperature in promoting and inhibiting seed germination is well documented and responses are highly variable among species. Heat stress plays a role in delaying germination in some species (Kepczynska et al. 2006; Norsworthy & Oliveira 2006) and long exposures to high temperatures can induce secondary dormancy (Nascimento et al. 2000). For other species, warm stratification reduces dormancy or promotes germination (Leon & Owen 2003; Turner et al. 2006). Cold stratification (Baskin & Baskin 2001; Moyo et al. 2009; Han & Long 2010) and oscillating temperatures (Baskin & Baskin 2001, 2003) have also been shown to break dormancy and/or promote germination.

Temperature can also have profound effects on the light sensitivity of seeds (Hilton 1984) and may play an important role in regulating seasonal germination responses (Heschel et al. 2007). Fluctuating temperatures can also overcome far-red light-induced inhibition of germination (Benvenuti et al. 2001; Honda & Katoh 2007). This response likely functions as an ecologically important gap-sensing mechanism by which seeds sense openings in the vegetative canopy (Honda & Katoh 2007); bare soil surfaces are expected to be less insulated from temperature fluctuations than a patch of soil covered with dense vegetation. As seeds that are dispersed to a low-competition safe site (e.g. bare soil) may still be exposed to far-red rich-filtered light from tree canopies, fluctuating temperatures may be a more reliable gap indicator. Seasonal regulation of germination timing may also be influenced by conditional dormancy. Conditionally dormant seeds are able to germinate under some temperatures, but not under the widest possible range of temperatures (Baskin & Baskin 2001), resulting in enhanced germination in some seasons or simulated seasonal temperatures (Kettenring & Galatowitsch 2007).
Although the Orchidaceae is the largest plant family and accounts for a large proportion of the biodiversity of some ecosystems, little is known about the role seasonal temperature plays in regulating orchid seed germination. The germination-promoting effects of pre-chilling on orchid seeds have been well documented (Rasmussen 1992; Miyoshi & Mi 1998; Shimura & Koda 2005; Kauth et al. 2011), indicating that germination timing is at least partially regulated by physiological dormancy. In addition, fluctuating temperatures were found to stimulate germination of Calopogon tuberosus var. tuberosus more than constant temperature (Kauth et al. 2011). Such investigations have important implications for the propagation of species for both ex situ and in situ conservation programs and as a means of revealing something about the poorly understood nature of orchid seed germination in situ. Although propagation is a key goal of many ex situ orchid conservation programs, an understanding of orchid seed physiology is rarely used to guide propagation practices. Few reports address the effects of temperature on orchid seed germination, but rather focus on the effects of culture media, plant growth regulators and other media additives on germination and seedling production. This emphasis on production has resulted in few physiological investigations of how germination is regulated in situ. In situ ‘seed baiting’ studies have been used to examine fungal specificity, fungal distribution, seed bank longevity and time to germination (Masuhara & Katsuya 1994; McKendrick et al. 2000; McKendrick et al. 2002; Whigham et al. 2006; Hollick et al. 2007), but the many confounding factors and course timescales make such studies less than ideal for understanding how temperature affects germination.

To study the effects and interactions of temperature and illumination on orchid seed germination, we carried out an asymbiotic seed germination experiment using the pine pink orchid, Bletia purpurea, from Florida as a model organism. Bletia purpurea is a terrestrial species found in the new world tropics and subtropics (Sosa & Díaz-Dumas 1997; Palestina & Sosa 2002). In North America, the species is found throughout Mexico, but is restricted to Florida in the USA where it is listed as state threatened (Coile & Garland 2003). Bletia purpurea occupies several different habitats in Florida and throughout its range. It can be found in dry or mesic habitats, in or along scrub lands or pinelands, on floating logs or stumps in cypress swamps and along highly disturbed lake edges and cliffs (Correll 1978; Dressler 1993; Williams & Allen 1998; Brown 2005).

The objectives of the present study were to determine the effect of simulated South Florida seasonal temperatures on B. purpurea seed germination and early seedling development and to determine whether oscillating temperatures promote germination under asymbiotic culture conditions. An additional objective was to determine whether there was an interaction between the effects of temperature and illumination (continual darkness compared with a 12 h photoperiod) on germination and development.

Materials and methods

Five undehisced, nearly mature B. purpurea capsules were collected from a population of plants at the Florida Panther National Wildlife Refuge (FPNWR; Collier County) Burn Unit 6 on 23 May 2007. The capsules were stored over Drierite desiccant at 22 ± 2°C for 3 weeks until the capsules were thoroughly dry and/or dehisced. Seeds were removed from the capsules, pooled and homogenized and then placed into cold storage at −10°C over Drierite (W.A. Hammond, Xenia, OH, USA) desiccant in the University of Florida Orchid Seed Bank.

The seeds were surface sterilized for 60 s in a solution of 6% Clorox bleach : 100% ethanol : distilled deionized (dd) water (1:1:20), then rinsed three times in sterile dd water. The seeds were then sown onto 9.0 cm diameter Petri plates containing 25 mL sterile Vacin and Went Modified Orchid Media (V895; PhytoTechnology Laboratories, Shawnee Mission, KS, USA; Vacin & Went 1949) amended with 1 g/L activated charcoal (Dutra et al. 2008). Approximately 30–50 seeds (41 ± 12; average ± standard deviation [SD]) were sown onto each plate. Plates were sealed with a single layer of NescoFilm and randomly assigned a temperature and illumination treatment.

Petri plates were cultured in growth chambers under one of five different temperature regimes. Four of these treatments were oscillating 12/12 h (day/night) temperature regimes designed to approximate seasonal day/night temperature fluctuations in central Florida. The experimental temperatures tested were 22/11, 29/19, 33/24 and 27/15°C (representing winter, spring, summer and autumn temperature extremes in south Florida, respectively), as well as a constant temperature treatment of 25°C. Constant, or near constant, temperatures in the range of 20–25°C are often used for orchid seed germination studies (see for example Pedroza-Manrique & Mican-Gutierrez 2006; Stewart & Kane 2006; Yamazaki & Miyoshi 2006; Lauzer et al. 2007; Dutra et al. 2009; Stewart & Kane 2010). Seeds were also treated with either continuous darkness or a 12/12 h light/dark (L/D) photoperiod at these various temperatures. Illumination was provided by cool white fluorescent lights at ~50 μmol/m²/s.

A completely randomized design (CRD) was used for this experiment with four replicate plates for all treatments. The experiment was repeated once (n = 8). Seeds and seedlings were observed after 3 weeks and 6 weeks of culture. At these times, data were collected on germination and subsequent leaf development (seedlings reaching one
leaves, two leaf and three leaf stages). Seeds were counted as germinated once the testa was cracked by the imbibed embryo. Percentage germination and the percentages of seedlings in each leaf stage were arcsine transformed prior to statistical analysis. An ANOVA and least square (LS) mean separation at \( \alpha = 0.05 \) was carried out using PROC MIXED in SAS v 8.02 (SAS Institute 2002).

### Results

Seeds exhibited high germination percentages (nearly 100%) within 6 weeks, regardless of the treatment. However, low temperatures and illumination delayed both germination and development (Figs. 1, 2). At week three, seeds cultured under illumination exhibited significantly lower germination than seeds in darkness (Table 1; Fig. 1). Interestingly, there was no significant difference between illumination treatments at constant 25°C or 33/24°C. Temperature and the interaction between the main effects of temperature and illumination also had a significant effect on germination at week three (Table 1; Fig. 1), with the lowest germination observed at simulated winter temperatures (22/11°C). After 6 weeks of culture, germination was not significantly affected by illumination, temperature or the interaction between these main effects (Table 1).

After 3 weeks of culture no seedlings had produced more than one leaf in the dark treatments, whereas a small percentage of seeds cultured in light at 25, 29/19 and 33/24°C produced a second leaf (\( \approx 1.5\% \); Fig. 2). At this time the percentage of seedlings producing one and two leaves was significantly affected by illumination (Table 1), with a pronounced developmental lag in the production of the first leaf observed for dark-treated seeds at 22/11°C. First leaf production was enhanced in light with increasing temperature treatments, although significant differences were not detected between the 25°C and 33/24°C treatments. After 6 weeks of culture, temperature, illumination and the interaction between temperature and illumination had significant effects on the percentage of seedlings in all three leaf stages. Culturing seedlings in the dark at 22/11°C resulted in significantly more seedlings developing one leaf and significantly fewer seedlings developing two leaves than other dark treatments. Among the dark treatments, the greatest percentage of seedlings developing two leaves was observed in seeds cultured at 33/24°C. No significant differences in the percentage of seedlings developing three leaves were detected in the dark treatments. Culturing seeds in light at 22/11°C resulted in significantly more seedlings developing one leaf and significantly fewer developing two leaves than other light treatments, as was observed among the dark treatments. Higher oscillating temperatures resulted in a greater proportion of seedlings transitioning from the one leaf stage to the two and three leaf stages. However, the greatest percentage of seedlings developing three leaves was observed at constant 25°C.

### Discussion

Bletia purpurea seeds germinated and underwent leaf differentiation under a wide range of temperature regimes in both darkness and light. Germination was slower when seeds were cultured at lower temperatures, although final germination was not affected. This initial delay in germination and development at the lower temperatures was greater in light than in dark. The lag was greatest at the lowest tested temperature regime and may serve an ecological function by delaying germination and development if seeds are near the soil surface when frost is likely.

The ability of B. purpurea to germinate maximally over a wide range of simulated seasonal temperatures may be due to its persistence in tropical and subtropical origins. In this environment the growing season is not limited by prolonged seasons of freezing winter temperatures and...
frost risk is typically fleeting. As the risk of fatality by freezing is relatively low, it may be that there is little adaptive advantage to relying on temperature cues for germination, particularly if other factors are more important for successful germination. Further examination of the roles that soil water potential, light quantity and quality, and seasonal effects on mycorrhizal fungal activity may play will elucidate when germination is most

![Graph showing effect of temperature and illumination on seedling development after 3 weeks and 6 weeks of culture. Error bars represent the standard error. Within each graph, bars from the same developmental class with the same letter are not significantly different at α = 0.05.](image)

Table 1 ANOVA results for the effects of temperature and illumination on germination and seedling development

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
<th>d.f.</th>
<th>Week 3 F</th>
<th>Week 3 P</th>
<th>Week 6 F</th>
<th>Week 6 P</th>
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<td>Germination</td>
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<td>Temperature</td>
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<td>0.36</td>
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<td></td>
<td>Interaction</td>
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<td>7.49</td>
<td>&lt; 0.01</td>
<td>0.57</td>
<td>0.69</td>
</tr>
<tr>
<td>First leaf stage</td>
<td>Illumination</td>
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<td>100.92</td>
<td>&lt; 0.01</td>
<td>13.42</td>
<td>&lt; 0.01</td>
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<td>&lt; 0.01</td>
<td>92.58</td>
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<tr>
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<td>&lt; 0.01</td>
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<td>&lt; 0.01</td>
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<td>&lt; 0.01</td>
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<tr>
<td>Third leaf stage</td>
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<td>–</td>
<td>–</td>
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<td>&lt; 0.01</td>
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<td>–</td>
<td>7.63</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

F-values in bold are significant at P = 0.05
likely to occur in nature, allowing managers to avoid common management practices that would harm delicate seedlings (i.e. mowing and burning) in order to promote establishment of this state threatened species.

Germination and development in light and dark was more rapid at constant 25°C compared with oscillating temperatures, except in light at 33/24°C. This is in contrast to what has been reported for other orchids (Kauth et al. 2011) and many non-orchids (Thompson & Grimes 1983; Probert et al. 1986), in which fluctuating temperatures have been shown to enhance germination. However, Probert et al. (1985) found variability in the degree to which Dactylis glomerata seeds from different regions in Europe responded to fluctuating temperatures and concluded that germination was enhanced by fluctuating temperatures in accessions with stronger initial dormancy, whereas germination of less dormant accessions was not significantly affected by fluctuating temperatures. Similarly, in a multi-region study of Calopogon tuberosus var. tuberosus populations in the USA, Kauth et al. (2011) found that populations that required longer chilling requirements to reach maximum germination also benefited most from fluctuating temperature regimes. This conclusion indicates that fluctuating temperatures are more important to species with physiological dormancy. In the absence of physiological dormancy, such as with B. purpurea, fluctuating temperatures may expose seeds to temperatures above or below an optimal temperature, resulting in delays to germination and development.

Simulated winter temperatures were found to delay germination and development more than higher temperatures. Bletia purpurea populations at the FPNWR are at the northern extent of their range and may be better adapted for warmer temperatures. Alternatively, delayed germination at the lowest tested temperature may be an adaptation to delay germination during winter when frosts are possible. The stronger inhibition of germination and development under illuminated conditions could be mediated by changes in the activity of different temperature-dependent phytochromes (Heschel et al. 2007). Delayed germination in light at 27/15°C and 29/19°C could be regulated similarly. A cold-induced negative photoblastic response may protect seeds from germinating and developing at the soil surface when temperatures are low and frost is more likely.

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References

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