Influence of mycorrhizal fungi on seed germination and growth in terrestrial and epiphytic orchids

Sameera A. Alghamdi
Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box 42805, Jeddah 21551, Saudi Arabia

Article info
Article history:
Received 1 August 2017
Revised 22 September 2017
Accepted 12 October 2017
Available online 13 October 2017

Keywords:
Epiphytic and geophytic orchids
Mycorrhiza
Light
Photosynthesis
Germination and establishment
Mycoheterotrophy
Autotrophy

Abstract
Epiphytes constitute over 70% of orchid diversity, but little is known about the functioning of their mycorrhizal associations. Terrestrial orchid seeds germinate symbiotically in soil and leaf litter, whereas epiphytic orchids may be exposed to relatively high light levels from an early stage of development and often produce green seeds. This suggests that seedlings of the two groups of orchids may differ in their responses to light and requirements for mycorrhiza-supplied carbon. The interactive effects of light, exogenous carbon and mycorrhizal status on germination and growth were investigated in vitro using axenic agar microcosms for one tropical epiphyte and three geophytic orchid species. The geophytic species strongly depended on their mycorrhiza for growth and this could not be substituted by exogenous sucrose, whereas the epiphytic species achieved 95% of the mycorrhizal seedling volume when supplied with exogenous sucrose in the dark. Mycorrhiza status strongly interacted with light exposure, enabling germination. Light inhibited or severely reduced growth, especially for the terrestrial orchids in the absence of mycorrhiza. For the first time, this study showed the parallel ecological importance of mycorrhizal fungi in overcoming light inhibition of seed germination and growth in both terrestrial and epiphytic orchids.

© 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The family Orchidaceae is the largest family of flowering plants comprising of approximately 32,000 species in around 800 genera with an average of a hundred discoveries of new species are made each year (Arditti, 1992). As well as being specious, orchids are ecologically very successful, being found in the majority of terrestrial ecosystems; from temperate grasslands to tropical rain forests (Moore et al., 1980; Arditti and Ghani, 2000) reflecting their extraordinary success in dispersing and adapting to a wide variety of habitats and environments. Two specialised groups have been recognised within family; the terrestrial orchids, which germinate underground in soil and represent around 25% of species, widely distributed in forest and grassland biomes, and the epiphytes, which use other plants for mechanical support growing on branches within their canopies, constituting at least 70% of all known orchid species (Ackerman, 1983) and are found mainly in tropical forests. The epiphytic habit has selected for a remarkable array of anatomical and physiological adaptations in orchids, including modified roots and photochemistry. Epiphytic orchid roots are modified to enable plants to anchor to their hosts, absorb episodic pulses of water and nutrients using a velamen sheath, a sponge-like material adapted to intercept canopy through-flow (Whitford, 1906) and in many cases, for the roots themselves to be green and photosynthetic. In extreme cases, some orchids, such as Harrisella porrecta, have abandoned leafy-shoot production altogether, and rely exclusively on their roots for photosynthesis (Benzing et al., 1983). The typical epiphyte shoot has thick leaves that permit water storage (Lütig, 1989), and many epiphyte species, for example Grammatophyllum speciosum and Bletia purpurea, engage in crassulacean acid metabolism (CAM) as adaptations to water limitation (Heitz et al.,1999; Khampa et al. 2010; Johnson et al. 2011).

In the early stages of their life cycle, all terrestrial orchids are non-photosynthetic, totally lacking chlorophyll and relying on carbon (C) acquired from a fungal symbiont for growth until the production of the first green leaves above ground, a nutritional strategy termed mycoheterotrophy (Leake 1994; Leake and Cameron, 2010). This is underpinned by the prodigious production...
of thousands of tiny seeds per plant containing minimal carbon and nutrient reserves for germination and growth leaving them totally dependent on a mycorrhizal fungal partner for the provision of C and mineral nutrients (Arditti, 1979). After initiation of the symbiosis, each embryonic protocorm develops into a protocorm, a specialised organ that defines the first stage of germination (Fig. 1). In in vitro culture, some orchid species can be grown asymptotically when sugars, amino acids and vitamins are exogenously provided in culture media, however, it is assumed these are normally obtained from the fungal partners in nature (Read, 1991; Arditti and Ghani, 2000; Bayman et al. 2002). With the exception of 1% of species that remain fully mycoheterotrophic throughout their life cycle (Leake, 1994), terrestrial orchids eventually emerge from the soil after months or even years, synthesise chlorophyll and become photosynthetic. In terrestrial orchids the autotrophic adult phase mycorrhiza can be important in uptake of mineral nutrients such as phosphorus (Alexander and Hadley, 1985; Cameron et al., 2006).

In contrast, the functional importance of mycorrhiza through the life cycles of epiphytic orchids are less well known, and may be distinct from that in terrestrial orchids, especially during germination since epiphytic orchids develop in the canopy and are thus exposed to relatively high light levels from an early stage of development (Leake and Cameron 2012). Recent studies of the fungal associates of over 150 tropical orchid species (Martos et al. 2012) have revealed a major ecological barrier between above- and belowground mycorrhizal fungal networks, with only about 10% of the fungal taxa partnering both epiphytic and terrestrial orchids, raising the hypothesis that there are important functional differences in mycorrhiza between the two kinds of orchid related to their distinct habitats (Leake and Cameron, 2012). Light intensity during seedling germination may play a role in this. The optimum light requirement for growth varies between epiphytic as well as terrestrial orchid species (Heifetz et al., 2000). In some terrestrial orchid species such as Goodyera repens even low levels of light inhibit germination, whereas other species such as Dactylorhiza purpurella, germination appears to be insensitive to light. Some studies have found that light can stimulate germination and early seedling establishment in the absence of exogenous carbon (Downie, 1941; Smith, 1973; Harvais, 1974).

Despite these early investigations, there remains no comparative study that investigates the role of light, and exogenous carbon supply of the germination and early development of geophytic and epiphytic orchids. In this study, we investigate effects of light, exogenous carbon and colonisation by mycorrhizal fungi on four orchid species studied in vitro for 56 days using the terrestrial species Goodyera repens, Dactylorhiza fuchsii and Orchis mascula and the epiphytic orchid Encyclia phoenicea. The hypothesis included: (a) germination rate and protocorm formation by all orchids would increase when germinated in the presence of a fungal symbiont or exogenous carbon (supplied as sucrose) and (b) light will significantly reduce the germination and growth of geophytic orchid seeds but enhance the germination and growth of the epiphytic species.

2. Material and methods

2.1. Plant and fungal material

Three temperate geophytic orchid species were selected, G. repens, O. mascula and D. fuchsii. These species range in their habitat requirements from being exclusively being found in shaded forests to growing in a range of ecosystems from woods to scrub, fens and short grassland in full sun. The epiphytic orchid, E. phoenicea, which grows throughout tropical areas of Mexico, Cuba and other islands in the Caribbean, typically grows on tree branches, close to sea level.

Seed capsules of G. repens were collected from beneath a stand of Pinus sylvestris at Tentsmuir Forest, Fife, UK, D. fuchsii capsules were collected from a grassland in the Rivelin Valley, Sheffield and O. mascula capsules were collected from a grassland at Lathkill Dale, Derbyshire, UK. Encyclia phoenicea, was supplied by Phil Sexton, Kew Gardens, London, UK. Cultures of mycorrhizal fungi for each geophyte species were obtained from intracellular hyphal

---

Fig. 1. Schematic representation of the life cycles of epiphytic and terrestrial orchids.
pelotons isolated from surface-sterilized pieces of orchid root and transferred aseptically to Petri dishes containing non-nutrient agar (Plant agar; Duchefa, Haarlem, the Netherlands). Vegetative hyphae growing from these pelotons were sub-cultured onto Fungal Isolation Medium (Table A) to obtain pure cultures for long-term storage at a constant temperature of 18 °C (Clements et al., 1986). Prior to use in this experiment, new cultures were established on non-nutrient plant agar from these stock plates.

2.2. Testing seed viability

Prior to the study, all seed were subjected to 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich UK, Gillingham) vital staining to investigate viability. The seeds were placed in five replicate small mesh bags and treated with 4% (w/v) calcium hypochlorite solution for 10 min to weaken the testa. The seeds were then put in sterile water for 24 h in darkness. They were then treated with 2% (w/v) tetrizolium, the pH of tetrizolium solution was adjusted to 7 and then left for 24 h in darkness. Seeds were examined under a microscope; seeds containing embryos by red staining were considered viable while unstained (white) embryos were considered non-viable (Van Waes and Debergh, 1986). Seed from all four species were at least 50% viable and thus included in the study.

2.3. Experimental microcosms

One hundred and twenty plates were prepared for each of the four species in 9 cm diameter vented Petri dishes containing 50 ml of either Rorison’s nutrient agar (for geophytes, Table B) of Knudson’s C nutrient agar (for the epiphyte, Table C). Forty of these plates contained sucrose at a concentration of 1 g L⁻¹, the remainder exposed to the light (see Table 1 for details). The length and breadth of ten embryos per dish (Fig. 2) were measured after 14, 28, 42 and 56 days under the light microscope (Nikon Dissecting Microscope coupled to a Leica fibre optic light source-CLS150).

Table A
Composition of fungal isolation medium (FIM) (based on Clements et al., 1986) as in 1L of nutrient solution.

| Compound       | g L⁻¹  |
|----------------|--------|
| NaNO₃          | 0.3    |
| KH₂PO₄         | 0.2    |
| MgSO₄·7H₂O     | 0.1    |
| KCl            | 0.1    |
| Yeast extract  | 0.1    |
| Sucrose        | 5.0    |
| Plant agar     | 10.0   |

Dishes were either maintained in the light in growth cabinets (conditions as above). Dishes were either maintained in darkness (light excluded from dishes with aluminium foil) or in the light in growth cabinets (conditions as above). Dishes were viewed 2, 4, 6 and 8 days post inoculation and the diameter of the resulting colony recorded across two axes at right angles to one another. The average diameter per dish was then calculated.

2.4. Fungal growth rates

Fungal isolates for all species described above were used to inoculate 9 cm diameter vented Petri dishes containing 50 ml of nutrient-free, water agar (N = 5). Dishes were either maintained in darkness (light excluded from dishes with aluminium foil) or in the light in growth cabinets (conditions as above). Dishes were viewed 2, 4, 6 and 8 days post inoculation and the diameter of the resulting colony recorded across two axes at right angles to one another. The average diameter per dish was then calculated.

Table B
Composition of 1/10 strength Rorison’s mineral solution (based on Hewitt, 1966) as in 1L of nutrient solution.

| Compound         | mg L⁻¹  |
|------------------|---------|
| CaCl             | 29.35   |
| MgSO₄·7H₂O       | 24.73   |
| K₂HPO₄·3H₂O      | 22.76   |
| Fe EDTA          | 1.97    |
| MnSO₄·4H₂O       | 0.20    |
| H₂B₄O₇          | 0.29    |
| Na₂MoO₄·2H₂O     | 0.025   |
| ZnSO₄·7H₂O       | 0.044   |
| CuSO₄·5H₂O       | 0.039   |

Table C
Knudson C modified orchid medium (based on Knudsen, 1946) as in 1L of nutrient solution.

| Compound         | mg L⁻¹  |
|------------------|---------|
| (NH₄)₂SO₄        | 500.00  |
| MgSO₄·7H₂O       | 122.12  |
| K₂HPO₄·3H₂O      | 250.00  |
| FeSO₄·7H₂O       | 25.00   |
| MnSO₄·4H₂O       | 5.682   |
| H₂B₄O₇          | 0.056   |
| Ca(NO₃)₂·4H₂O    | 694.4   |
| ZnSO₄·7H₂O       | 0.331   |
| CuSO₄·5H₂O       | 0.0624  |

2.5. Statistical analysis

Differences between treatment means of seedling volume were analysed by two-way ANOVA followed by Tukey’s multiple comparison test (Minitab release 15) at P < .05. Analysis of variance was performed using Log₁₀-transformed data; untransformed means are presented in all cases.

\[ V = \frac{\pi b^2 l}{6} \]

where \( V \) = volume (mm³), \( l \) = length (mm) and \( b \) = breadth at the widest point (mm). See Fig. 1 for definitions.
3. Results

Across all species, the effect of the presence of fungus or sucrose on germination was more pronounced when seeds were cultured in darkness (Table 2), however, the magnitude of these responses varied with orchid species. The three factors (no fungus, no fungus + sugar and + fungus) as well as light and growth period significantly increased G. repens seed germination (df = 2, 117; F = 166.72; P < .001). Seedlings of G. repens that were germinated with their mycorrhizal fungus experienced higher germination in the dark whereas those with sucrose only showed limited germination, even when cultured in darkness. The seeds cultured with fungus in the light gave lowest germination rate and did not develop significantly over the 56-day growth period (Fig. 3, Table 2). Almost all of the G. repens seeds that were in contact with the mycorrhizal fungus and cultured in darkness germinated within two weeks, whereas those that were supplied with sucrose in the dark did not begin germinating until three weeks after imbibition. Testa rupture occurred at 28 days, and by day 42, rhizoids were observed. On day 56, the apical meristem had developed in mycorrhizal plants in darkness, but it lacked chlorophyll. Germination and early development was limited when seeds were cultured in darkness without the mycorrhizal fungus. Growth of seedlings of this species was strongly inhibited by light in the absence of the fungal partner (Fig. 3, Table 2).

Both light and carbon supply significantly affected the rate of germination of D. fuchsii (df = 2, 119; F = 1.97; P < .001). With sucrose, D. fuchsii germination was stimulated after two weeks in the dark (Fig. 4, Table 2), but the greatest growth was achieved by mycorrhizal plants by 56 days. Poorest growth occurred in the light in the absence of the fungus or sucrose (Fig. 4). D. fuchsii seeds were able to germinate under both light and dark conditions although their growth in the light was very slow. In contrast to G. repens, the fungus did not substantially enhance orchid germination in the light although growth of these plants was significantly greater then those grown without the fungus (Fig. 4).

There was no germination or growth of O. mascula in the light in any treatment (Fig. 5, Table 2). In the dark, this species showed low rates of germination and growth across all treatments (Fig. 5). O. mascula achieved its best growth in the dark with a fungal partner. Sucrose was unable to fully replace the benefits of symbiotic germination (df = 2, 119; F = 493.25; P < .001) (Fig. 5, Table 2).

Light and fungus/carbon treatments had highly significant interaction effects germination of E. phoenicea (ANOVA: df = 2, 59; F = 113.63; P < .001) (Table 2). With fungus, seeds started to increase in size after 14 days and the testa ruptured by 28 days, irrespective of darkness or light treatment, although light exposure slightly reduced the rate of seedling development. In contrast, under asymbiotic conditions germination and seedling development was strongly inhibited by light, even in the presence of sucrose (Fig. 6). Seedling development was extremely limited when seeds were cultured in light without a fungal or sugar source of carbon.

Exposure to light lead significantly reduced the radial growth rates of the fungal symbionts of G. repens and D. fuchsii over 8 days (Fig. 7, Table 3). These fast growing fungi grew across the full area of the agar plates within 8 days, whereas the fungal symbiont from O. mascula grew much more slowly, but nonetheless was also found to be inhibited by light (Fig. 7, Table 3).

Table 2
Two-way ANOVA table comparing the volume four species of orchid seedling after 56 days of in vitro culture.

| Source         | G. repens | D. fuchsii | O. mascula | E. phoenicea |
|----------------|-----------|------------|------------|--------------|
| Carbon¹        | df 2      | 2          | 1          | 2            |
| Light          | 1         | 3353       | 343        | 561          |
| Carbon * Light | 2         | 166        | 1.97       | 453          |
| Total          | 117       | 119        | 119        | 59           |

¹ Three levels: no carbon (other than that present in the agar), exogenous sugar supplied as sucrose or inoculation with the appropriate fungal symbiont.
4. Discussion

In nature, most orchids are critically reliant upon fungi for germination, establishment and subsequent growth as they are lacking in sufficient seed reserves for germination. This relationship, while a key feature of soil-dwelling terrestrial orchid species that produce achlorophylous seedlings, termed protocorms. These terrestrial protocorms are totally reliant on fungi for the provision of carbon and mineral nutrients (Leake and Cameron, 2010) but the role of mycorrhizal fungi in epiphyte germination is less clear. Indeed it has been suggested that mycorrhizas may less important for germination and development of epiphytic orchid species which do not develop in soil, are exposed to light in the canopy at an early stage and are often able to produce green, and presum-
ably photosynthetic, protocorms. Recently, intense fungal coloni-
sation has been recognised in epiphytic species growing in the aer-
ial environment (Martos et al., 2012) leading to the suggestion that
this symbiosis may be more important in the epiphytic environ-
ment than first thought (Leake and Cameron, 2012). In order to
understand the dynamics of this complex relationship, a number
of studies have focused on in vitro asymbiotic and symbiotic germi-
nation of orchids but they ignore the important interaction
between light and carbon supply in regulating germination;
whether seed germination is influenced by light/dark treatments
and standardisation of these germination conditions used across
a range of studies is seldom indicated. Given the vital role of fungi
and the potential for environmental modulation of orchid germina-
tion, there is an urgent need to ascertain whether the relationship
between the fungal symbiont and the plant is critical for germina-
tion and growth in early stages. Indeed, orchid conservation is

Fig. 5. Effect of light and carbon supply on the asymbiotic and symbiotic O. mascula seeding volume. Error bars represent one standard error of treatment means. Within each graph, differences between mean volumes at 56 days were resolved using 2-way ANOVA followed by Tukey’s multiple comparison test; bars with differing letter are significantly different at \( P < .05 \) (Table 2).

Fig. 6. Effect of light and carbon supply on the asymbiotic and symbiotic E. phoenicea seeding volume. Error bars represent one standard error of treatment means. Within each graph, differences between mean volumes at 56 days were resolved using 2-way ANOVA followed by Tukey’s multiple comparison test; bars with differing letter are significantly different at \( P < .05 \) (Table 2).
dependent on such data for ex-situ conservation and reintroduction projects (Ramsay and Stewart, 1998).

The present study examines the impact of different light and carbon source on the rate of germination and early seeding development over a time course in three terrestrial orchid species *G. repens*, *D. fuchsii* and *O. mascula* and one species of epiphytic orchid, *E. phoenicea*. The dependence of orchids on mycorrhizal fungi can be highly variable from species to species, thus it is possible that epiphytic orchids may not be dependent on mycorrhizal fungi throughout all their life cycle, especially at the earliest stages, whereas terrestrial orchids remain mycorrhizal throughout their life cycle. Indeed it is likely that in adult orchids, mycorrhizas supply nutrients, especially nitrogen and phosphorus, in return for carbon, this having been proven for *G. repens* (Cameron et al., 2006, 2007, 2008).

Based on these differential life histories, I hypothesised that geophyte germination would be greatest in the dark in the presence of fungi and lowest under irradiated conditions in the absence of the fungus. Even in symbiosis, germination in the light was not only reduced in absolute terms, but delayed in initiation with symbiotic seeds cultured in darkness germinating within the first 14 days post-imbibition but symbiotic seeds cultured in the light not germinating until 42 days post imbibition. A similar pattern was observed for the other two terrestrial species studies; although *D. fuchsii* seeds were able to germinate under all conditions, the greatest germination and subsequent growth was achieved symbiotically in darkness with light and the absence of the fungal symbiont acting to dramatically reduce germination and growth. Again, *O. mascula* did not germinate in the light and the presence of its fungal symbiont enhanced germination and growth over those individuals supplied with exogenous carbohydrate. In direct contrast, the epiphytic orchid, *E. phoenicea*, germinated readily in the dark, both with and without the fungal symbiont, although germination was enhanced in this asymbiotic state by the provision of sugar in the culture medium. While, in the light, germination was reduced to a quarter of the levels achieved in the dark when grown asymptomatically. When grown with its fungal partner in the light however, germination and subsequent growth was restored to levels comparable with those individuals grown in the dark, a situation also seen in other orchid species (Johnson et al., 2007, 2011). This observation could be underpinned by a range of factors including direct modulation of plant metabolism through bilateral signalling between symbiotic partners or by the enhanced nutrition of symbiotic individuals grown in the light facilitating enhanced photosynthetic activity via increased syntheses in photochemical pigments and proteins such as Rubisco (Fukai et al., 1997).

The underpinning mechanisms for this sensitivity of orchids to light remain unclear (Andrews, 1997; Baskin and Baskin, 1998). Inhibition via light signalling, possibly mediated by phytochromes or other photoreceptors known to play a role in the regulation of seed germination (Stewart and Kane, 2010) could represent candidate mechanism as they are regulate plant responses to red and far-red light which are moderated during seedling emergence (Bae and Choi, 2008; Sharrock, 2008) as well as the observation access of the plant to simple carbohydrates in the culture medium (Majerowicz and Kerbauy, 2002; Stewart and Kane, 2010). *G. repens* seeds did not germinate under irradiated conditions in the absence of the fungus. Even in symbiosis, germination in the light was not only reduced in absolute terms, but delayed in initiation with symbiotic seeds cultured in darkness germinating within the first 14 days post-imbibition but symbiotic seeds cultured in the light not germinating until 42 days post imbibition. A similar pattern was observed for the other two terrestrial species studies; although *D. fuchsii* seeds were able to germinate under all conditions, the greatest germination and subsequent growth was achieved symbiotically in darkness with light and the absence of the fungal symbiont acting to dramatically reduce germination and growth. Again, *O. mascula* did not germinate in the light and the presence of its fungal symbiont enhanced germination and growth over those individuals supplied with exogenous carbohydrate. In direct contrast, the epiphytic orchid, *E. phoenicea*, germinated readily in the dark, both with and without the fungal symbiont, although germination was enhanced in this asymbiotic state by the provision of sugar in the culture medium. While, in the light, germination was reduced to a quarter of the levels achieved in the dark when grown asymptomatically. When grown with its fungal partner in the light however, germination and subsequent growth was restored to levels comparable with those individuals grown in the dark, a situation also seen in other orchid species (Johnson et al., 2007, 2011). This observation could be underpinned by a range of factors including direct modulation of plant metabolism through bilateral signalling between symbiotic partners or by the enhanced nutrition of symbiotic individuals grown in the light facilitating enhanced photosynthetic activity via increased syntheses in photochemical pigments and proteins such as Rubisco (Fukai et al., 1997).

The underpinning mechanisms for this sensitivity of orchids to light remain unclear (Andrews, 1997; Baskin and Baskin, 1998). Inhibition via light signalling, possibly mediated by phytochromes or other photoreceptors known to play a role in the regulation of seed germination (Stewart and Kane, 2010) could represent candidate mechanism as they are regulate plant responses to red and far-red light which are moderated during seedling emergence (Bae and Choi, 2008; Sharrock, 2008) as well as the observation

---

**Table 3**

General Linear Model ANOVA table comparing the growth on orchid mycorrhizal in vitro culture without the associated fungal seedling over 8 days.

| Source                  | df | F      | P      |
|-------------------------|----|--------|--------|
| Fungal Species          | 2  | 3051   | <.001  |
| Light                   | 1  | 675.3  | <.001  |
| Time                    | 3  | 1502   | <.001  |
| Species * Light         | 2  | 144.8  | <.001  |
| Species * Time          | 6  | 363.0  | <.001  |
| Light * Time            | 3  | 106.4  | <.001  |
| Species * Light * Time  | 6  | 26.26  | <.001  |
| Total                   | 119|        |        |

---

**Fig. 7.** Effect of light on the growth of the fungal symbiont without the orchid protocorm over an 8 day period. Error bars represent one standard error of treatment means. Differences between mean colony diameter at 2, 4, 6 and 8 days were resolved using 3-way ANOVA (Table 3).
that the phytochrome spectrophotometric signal rapidly increases through seed imbibition as a result of rehydration of inactive phytochrome (Kendrick and Spruit, 1977). However, an allied hypothesis to explain reduced orchid germination under irradiated conditions lies with the basal physiology of the fungus itself.

I hypothesised that growth of the obligately heterotrophic fungus would be enhanced in conditions mimicking its natural habitat, in the soil or in epiphytic species, in accumulated organic material in on the tree surface, i.e., in darkness. Concurrent with the hypothesis, fungal growth was significantly reduced when cultured under continuous light, such reduced growth rate, and presumably impaired physiology, is one of the likely drivers of reduced germination of orchid seeds and growth of symbiotic seedlings in the light. To the contrary, the present asymbiotic seedlings grown in the light showed reduced growth relative to those grown in the dark. Moreover, the presence of the fungus enhanced seedling growth in the light when compared to co-specifics cultured asymbiotically, again under irradiated conditions. This observation is thus supportive of the notion of direct physiological inhibition of the juvenile orchid by light.

In conclusion, it is clear that the geophytic orchid seeds need to establish a relationship with fungus in the early stage of growth in order to obtain the necessary carbon to reach maturity at which point they photosynthesise. However, the epiphyte becomes photosynthetic at a much earlier life stage, prior to maturity and substantially before the formation of leaves. Presumably epiphytic species are less reliant on the fungus for the provision of C sooner in their lifecycle. The present observation that light leads to a weaker suppression of germination and growth in the epiphyte supports this hypothesis and suggests that this transition shifts the trophic status of the symbiosis from a primary role in provisioning the orchid with C to provisioning of nutrients. Given that the fungi that have studied varied in the magnitude of their lifecycle. The present observation that light leads to a substantial before the formation of leaves. Presumably epiphytic order to obtain the necessary carbon to reach maturity at which

References

Ackerman, J., 1983. On the Evidence for a Primatively Epiphytic Habit in Orchids. American Society of Plant Taxonomists (ASPT), vol. 8, p. 474–477.

Andrews, T.S., 1997. Factors affecting the germination of Giant Parramatta grass. Aust. J. Exp. Agric. 37, 439–446.

Arndt, J., 1979. Factors affecting the germination of orchids. Bot. Rev. 33, 1–97.

Arndt, J., 1992. Fundamentals of Orchid Biology. John Wiley & Sons, New York, p. 691.

Arndt, J., Chani, A., 2000. Tansley review, 110 – numerical and physical properties of orchid seeds and their biological implications. New Phytol. 143, 367–421.

Bae, G., Choi, C., 2008. Decoding of light signals by plant phytochromes and their interacting proteins. Annu. Rev. Plant Biol. 59, 281–311.

Baskin, J.M., Baskin, C.C., 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, California, USA.

Bayman, P., Gonzalez, E.J., Fumero, J.J., Trenblay, R.L., 2007. Factors affecting the germination of orchid seeds and their biological implications. New Phytol. 171, 405–416.

Bayman, P., Gonzalez, E.J., Fumero, J.J., Trenblay, R.L., 2007. A different kind of parasitic plant: a brief history of mycoheterotrophy and epi-parasitism. Haustorium 50, 4–6.

Cameron, D.D., Leake, J.R., Read, D.J., 2006. Mutualistic mycorrhiza in orchids: evidence from plant–fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid Goodyera repens. New Phytol. 171, 405–416.

Clements, M.A., Muir, H., Cribb, P.J., 1984. A preliminary report on the symbiotic germination of European terrestrial orchids. Kew Bull. 41, 437–445.

De Wit, D.G., 1941. Notes on the germination of some British orchids. Trans. Bot. Soc. Edinb. 33, 94–103.

Dutra, D., Johnson, T.R., Kauth, P.J., Stewart, S.L., Kane, M.E., Richardson, L., 2008. Asymbiotic seed germination, in vitro seedling development, and greenhouse acclimatization of the threatened terrestrial orchid Bletia purpurea. Plant Cell Tissue Organ Cult. 94, 235–245.

Fukai, S., Fujiwara, K., Okamoto, K., Hasegawa, A., Goi, M., 1997. Effects of red and blue light on germination and protocorm growth of Calanthe Satsuma. Lindleyana 12, 169–171.

Hewitt, E.J., 1966. Seed and water culture methods used in the study of plant nutrition. Commonw. Agric. Bureau Tech. Commun. 22, 190–191.

Johnson, T.R., Kane, M.E., 2007. Asymbiotic germination of ornamental Vanda: in vitro germination and development of three hybrids. Plant Cell Tissue Organ Cult 91, 251–261.

Johnson, T.R., Kane, M.E., Perez, H.E., 2011. Examining the interaction of light, nutrients and carbohydrates on seed germination and early seedling development of Bletia purpurea (Orchidaceae). Plant Growth Regul. 63, 89–99.

Kendrick, R.E., Spruit, C.J.P., 1977. Phototransformations of phytochrome. Photochem. Photobiol. 26, 201–214.

Klopp, S., Wangsomnuk, P., Wonsomnuk, P., 2010. Factors affecting seed germination of Grammatophyllum speciosum cultured in vitro. Asia Pac. J. Mol. Biol. Biotechnol. 18, 193–197.

Knudsen, L., 1946. A new solution for germination of orchid seeds. Am. Orchid Soc. Bull. 15, 214–217.

Leake, J.R., 1994. The biology of myco-heterotrophic (‘saprophytic’) plants. New Phytol. 127, 171–216.

Leake, J.R., Cameron, D.D., 2010. Physiological ecology of mycoheterotrophy. New Phytol. 185, 601–605.

Leake, J.R., Cameron, D.D., 2010. Untangling above and below ground mycorrhizal fungal networks in tropical orchids. Mol. Ecol. 21, 4921–4924.

Lüttge, U., 1989. Vascular Plants as Epiphytes: Evolution and Ecophysiology. Springer-Verlag Incorporated, Berlin, Germany.

Majerowicz, N., Kerbauy, G.B., 2002. Effects of nitrogen forms on dry matter and nitrogen content in vascular plant along an altitudinal transect. Plant Cell Environ. 22, 1435–1443.

Ramsay, M.M., Stewart, J., 1998. Re-establishment of the lady’s slipper orchid (Cypripedium calceolus L.) in Britain. Bot. J. Linn. Soc. 126, 173–181.

Read, D.J., 1991. Mycorrhiza in ecosystems. Experientia 47, 376–391.

Sharrock, R.A., 2008. The phytochrome red/far-red photoreceptor superfamily. Curr. Opin. Plant Biol. 11, 230–239.

Webb, D.A., 1980. Orchidaceae in Flora Europaea. Cambridge University Press, Cambridge, United Kingdom.