Green and blue light photoreceptors are involved in maintenance of dormancy in imbibed annual ryegrass (*Lolium rigidum*) seeds

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Summary

• Light plays an important role in two separate processes within the seeds of *Lolium rigidum* (annual ryegrass). Dormant seeds of *L. rigidum* remain dormant when imbibed in the light, but once seeds have lost dormancy through dark-stratification, light stimulates their germination. This study characterizes the light qualities and quantities which are effective in maintenance of dormancy.

• Dormant seeds were stratified under narrow- and broad-waveband light to identify the potential photoreceptors involved in dormancy maintenance, and to determine whether dark-induced dormancy loss is reversible by light.

• Blue and green light both mediated dormancy maintenance in a far-red-independent manner. Red light resulted in dormancy maintenance only when far-red wavelengths were excluded, suggesting a redundant function of phytochrome. At low fluence rates, white light was more effective than monochromatic light, suggesting the action of multiple photoreceptors in dormancy maintenance. By contrast, nondormant seeds did not germinate unless provided with red light.

• These results indicate that seed dormancy maintenance is potentially mediated through the actions of blue and green light photoreceptors. Seed dormancy could thus be added to the growing list of plant responses that may be mediated by green light in a cryptochrome-independent manner.

Key words: cryptochrome, dormancy, germination, green light, *Lolium rigidum*, phytochrome, seed.

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Introduction

Release of seed dormancy should be considered as a separate process from seed germination (Benech-Arnold *et al.*, 2000), and this is particularly apparent when the conditions required for dormancy release (light, temperature, etc.) are different from those required for germination. Germination, defined here as protrusion of the radicle through the testa, is achieved when the growth potential of the embryo exceeds the mechanical resistance of the endosperm (Nonogaki, 2006). A complex interaction of transcription factors, plant growth regulators and enzyme activities related to cell wall breakdown and energy metabolism determine whether, and at what time, the growth of the radicle is sufficient to penetrate the endosperm and testa (Finch-Savage & Leubner-Metzger, 2006). Depending on the type of seed and its state of dormancy, light-requiring seeds can be stimulated to germinate by an extremely short pulse of far-red or red light (mediated by phytochrome A); they may require exposure to red light for minutes or hours in a far-red-reversible response (mediated by phytochrome B in *Arabidopsis*); or germination can be inhibited by prolonged exposure to supplementary far-red light (probably mediated by phytochrome A, although this is not yet proven; reviewed in Casal & Sánchez, 1998). In contrast to the vast literature on

seed germination, the role of light in maintaining seed dormancy, as opposed to inhibiting germination, is relatively obscure.

When imbibed at warm (20–30°C) temperatures, dormant seeds of the weedy grass species *Lolium rigidum* remain dormant if kept in the light, but lose dormancy in the dark (Steadman, 2004; Steadman *et al.*, 2004). Like many weed species (Batlla & Benech-Arnold, 2005), nondormant *L. rigidum* seeds cannot germinate until transferred to light, demonstrating that dormancy release (which requires dark) and initiation of germination (which requires light) are separate processes in this species. Preliminary studies suggest that classical far-red-reversible phytochrome action is involved in the maintenance of dormancy, with seeds stratified under red light remaining dormant, and seeds stratified under far-red light losing dormancy (Steadman, 2004).

Plants possess three major, well-characterized photoreceptor classes (the phytochromes, cryptochromes and phototropins), as well as a putative green light receptor, which is now receiving more study as a result of the availability of *Arabidopsis* photoreceptor mutants. Phototropins absorb blue and ultraviolet light and are involved in phototropism, stomatal opening and chloroplast movement (Christie, 2007). The putative green light receptor(s) has been implicated in early stimulation of hypocotyl elongation in etiolated seedlings (Folta, 2004), down-regulation of light-responsive plastid genes (Dhingra *et al.*, 2006) and in mediation of shade avoidance in *Arabidopsis* rosettes (Mullen *et al.*, 2006). The members of the phytochrome family efficiently perceive red and far-red light, but are also capable of absorbing other wavelengths to some extent. Phytochrome A, the light-labile (type I) phytochrome, mediates responses to continuous far-red light, as well as to very dim or very brief flashes of light from all regions of the spectrum (Fankhauser, 2001). Recent studies have shown that phytochrome A can also exist in a stable, red light-activated form (Sineshchekov *et al.*, 2006; Franklin *et al.*, 2007). The best-studied light-stable (type II) phytochrome, phytochrome B, shows responses to red light which are usually fluence rate-dependent, obey the Bunsen–Roscoe law of reciprocity and are reversible by subsequent treatment with far-red light (Casal & Sánchez, 1998). The cryptochromes are activated by blue and ultraviolet light but do not perceive red or far-red light (Ahmad *et al.*, 2002). There is growing evidence that cryptochrome-mediated responses are blue : green reversible, analogous to the red : far-red reversibility of phytochrome B responses (Banerjee *et al.*, 2007; Bouly *et al.*, 2007).

The phytochromes and cryptochromes are involved in regulating many aspects of plant growth and development (Sullivan & Deng, 2003) and it is likely that they share a downstream regulatory mechanism for many photomorphogenic processes. Although there have been a number of studies on the inhibition of seed germination by blue light, mainly in horticultural species (Thanos & Mitrakos, 1992; Tozzi *et al.*, 2005), none has identified the photoreceptor(s) involved. However, Poppe *et al.* (1998) found that seeds of *Arabidopsis* mutants lacking the cryptochrome 1 gene were unable to germinate under blue or ultraviolet light, suggesting a role for cryptochrome in the promotion of *Arabidopsis* seed germination. By contrast, as outlined earlier, there is a vast collection of literature detailing the response of seed germination to various regimes of red and far-red light (Batlla & Benech-Arnold, 2005; Appenroth *et al.*, 2006).

The situation in imbibed, dormant *L. rigidum* seeds, in which dark-induced dormancy release is inhibited by white or red light (Steadman, 2004), has prompted this study to characterize the light qualities and quantities which are effective in dormancy maintenance in this species. There are no photoreceptor mutants of *Lolium* available, so careful characterization of the response to narrow- and broad-waveband light was performed to provide an indication of the photoreceptor(s) involved in maintenance of dormancy.

Materials and Methods

Seed material

Freshly matured *Lolium rigidum* Gaud. (annual ryegrass) seeds were collected from plants infesting a wheat field at Wongan Hills, Western Australia (30°53′S, 116°43′E) in October/November of 2000 and 2006, and stored in sealed foil pouches at −20°C in order to maintain their dormancy status. *L. rigidum* is an obligate outcrossing species so it is highly genetically variable (Powles & Preston, 2006); however, seeds collected from this particular field have consistently been more dormant than those from surrounding fields. Seed moisture contents, determined by measuring the mass of seeds before and after drying at 103°C for 24 h, were 13% (2000 population) and 15% (2006 population), and viability, tested by tetrazolium staining following the protocol of Steadman (2004), was close to 100%.

Light sources

Stratification treatments were carried out in a 20°C controlled-temperature room. Seeds were placed in lightproof boxes with a 130 × 130 mm window cut in the top face, 160 mm above the seeds. The window was either filled with an array of light-emitting diodes (LEDs) or covered with a Lee theatrical filter (Lamp Replacements, Perth, Australia) and illuminated with a 50 W halogen globe 360 mm above the filter. Light fluence rates and spectral composition were measured using a StellarNet EPP2000-VIS spectrometer with irradiance calibration (Warsash Scientific, Sydney, Australia). White (W) light was provided by unfiltered halogen globes. Broad-waveband red (R), blue (B) or green (G) light was obtained by filtering the halogen globes through Lee 106, 713 or 124 filters, respectively. Narrow-waveband R was provided by an array of 80 (8 × 10) 8 mm diameter LEDs (Futurlec, Broadmeadow, Australia; peak wavelength 654 nm, half-bandwidth 11 nm). Arrays of 25 (5 × 5) ultrabright 5-mm-diameter LEDs (Ultraleds, Macclesfield, UK) were used for narrow-waveband B (peak wavelength

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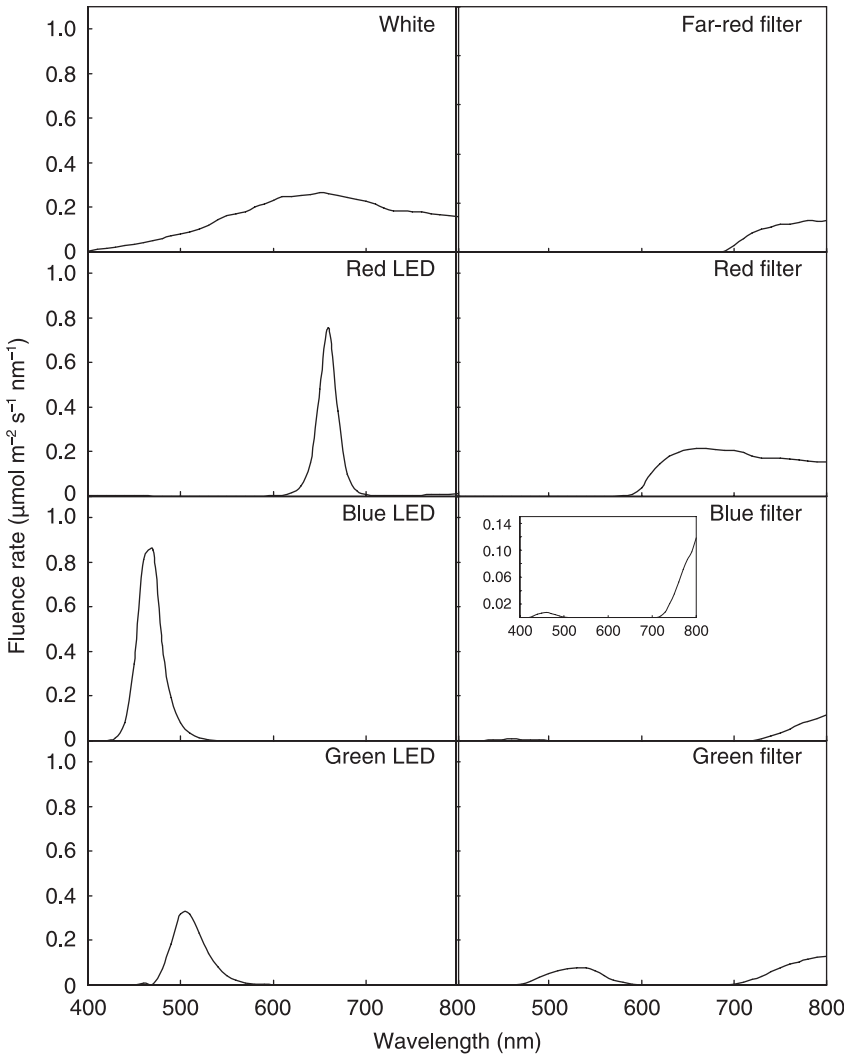

Fig. 1 Spectra for light sources used in experimental treatments. Spectral composition and fluence rates were measured for all light qualities during stratification of *Lolium rigidum* seeds. The spectrum for the blue filtered light is shown with an expanded *y*-axis in the inset.

465 nm, half-bandwidth 13 nm) and G (peak wavelength 504 nm, half-bandwidth 19 nm) light. Far-red (FR) light was obtained by filtering halogen globes through a combination of Lee 106 and Lee 120 filters (wavelength 700 nm and above). Spectra for these light sources are shown in Fig. 1. Lee 209 neutral density filters were used to decrease the fluence rates of the W and LED light when required. The temperature of the agar on which the seeds were sown ranged between 24 and 25°C in the boxes illuminated by halogen lights or LED arrays, depending upon the light quality and fluence rate.

Seed stratification and germination conditions

Dry seeds were placed in 90-mm-diameter Petri dishes containing 1% (w/v) agar dissolved in deionized water (50 seeds per plate, four replicates for each treatment) and stratified at a constant temperature of 20°C for 21 d under the appropriate light conditions. Following stratification, Petri dishes were transferred to a growth cabinet with a 12 h photoperiod and day : night temperatures of 25 : 15°C (hereafter referred to as 'germination conditions'). Light in the growth cabinet was provided by a bank of five fluorescent tubes and eight incandescent bulbs, with a combined fluence rate of 90 µmol m^{-2} s^{-1}. Following transferral of Petri dishes to germination conditions, seed germination was scored regularly for the next 24 d. Ungerminated seeds were gently pinched to determine if they contained a living, turgid embryo (pilot studies demonstrated that this gave the same results as tetrazolium staining), and dead or empty seeds were excluded from calculations. Each stratification experiment was accompanied by nonstratified (dishes placed directly in germination conditions) and dark-stratified (dishes kept wrapped in foil at 20°C until germination) controls.

Experimental design

Effect of light quality on seed dormancy Seeds were stratified for 21 d at 20°C in eight different light environments alongside dark-stratified controls, before transferral to germination conditions with the nonstratified control. Four identical

experiments, each with four replicates of each treatment, were carried out at different times, two with the seeds collected in 2000 and two with the seeds collected in 2006. Data from the four experiments were combined to obtain the overall mean germination percentage for each treatment. Light fluence rates (in μmol m^{-2} s^{-1}) during stratification were as follows: W, 44.6; R_{filter}, 18.3; R_{LED}, 22.8; B_{filter}, 0.3; B_{LED}, 28.1; G_{filter}, 5.5; G_{LED}, 14.8; and FR_{filter}, 0.2.

Effect of light quality on dark-stratified (nondormant) seeds Seeds from the 2006 population were stratified in the dark for 21 d at 20°C in order to induce dormancy release, and subsequently exposed to dark, W, R, FR, B or G for another 21 d, before finally being transferred to standard germination conditions. The second phase of stratification was carried out either under constant light at 20°C or under a 25 : 15°C 12 h day : night cycle. As it was not possible to construct LED arrays in the 25 : 15°C growth cabinet, all light qualities throughout this experiment were provided by the Lee filters. Light fluence rates for the constant 20°C treatments were the same as for the experiment on the effect of light quality on seed dormancy (see earlier); for the 25 : 15°C treatments, fluence rates were (in μmol m^{-2} s^{-1}) as follows: W, 84.2; R_{filter}, 25.3; B_{filter}, 2.7; G_{filter}, 15.1; and FR_{filter}, 0.6.

Effect of fluence rate on seed dormancy Neutral density filters (Lee 209) were used to reduce and approximately equalize the fluence of W, B_{LED}, G_{LED} and R_{LED} lights, producing three fluence rates (average value in μmol m^{-2} s^{-1}, with the exact fluence rate for each light quality following in square parentheses): (1) 6.2 [W, 5.4; B, 6.2; G, 6.7; R, 6.4]; (2) 2.7 [W, 2.5; B, 2.9; G, 3.2; R, 2.0]; (3) 0.3 [W, 0.3; B, 0.2; G, 0.3; R, 0.3]. In two identical experiments, each with four replicates, dormant seeds from the 2006 collection were stratified under these conditions for 21 d, alongside dark-stratified controls, before transferral to germination conditions with the nonstratified control. The data from the two experiments were combined.

Effect of light pulses on dark-induced dormancy release Experiments on photoreceptor responses often test the validity of the Bunsen–Roscoe law of reciprocity. This law is obeyed if a photoreceptor response is dependent upon the total number of photons delivered, but independent of the time over which they are applied (Warhepa *et al.*, 1989). Short-term studies on inhibition of hypocotyl elongation in dark-grown seedlings often employ light pulses of different number, duration and intensity such that the total fluence in each treatment is the same. The situation in dormant *L. rigidum* seeds is somewhat different, as long-term exposure to dark, not light, elicits the response of interest (i.e. dormancy release), which increases with increasing durations of dark-stratification (Steadman, 2004). However, an experiment was designed along the lines of a reciprocity test, to determine if interruption of dark-stratification with light pulses could inhibit dormancy release, and, if so, whether the duration or intensity of the light was most important. Seeds from the 2006 population were exposed to pulses of different fluence rates of R_{LED}, B_{LED} or G_{LED} every third day during 21 d of dark-stratification, such that each treatment (within the appropriate light quality) received the same total fluence. Details of the treatments are given in Table 1. In the first experiment, seeds were exposed, at four different fluence rates (designated (i)–(iv) in Table 1), to a total R_{LED} fluence of 5.4×10^5 μmol m^{-2}, a total B_{LED} fluence of 3.7×10^5 μmol m^{-2} or a total G_{LED} fluence of 5.0×10^5 μmol m^{-2}. In the second experiment, seeds were exposed, at three different fluence rates (i–iii), to a total R_{LED} fluence of 3.6×10^6 μmol m^{-2}, a total B_{LED} fluence of 5.3×10^6 μmol m^{-2} or a total G_{LED} fluence of 5.8×10^6 μmol m^{-2}. In both experiments, the lowest fluence rate was provided as constant light for 21 d. The total fluences and fluence rates used in these experiments were within the range of values commonly used in photomorphogenic and seed germination experiments. Following the 21 d stratification treatments, the seeds were transferred to germination conditions along with nonstratified and dark-stratified controls. Each treatment and control consisted of four replicates.

Statistical analyses

Data was analysed by one- or two-factor ANOVA at the 5% level of significance, and differences between pairs of treatment means were assessed by least significant difference tests.

Results

Effect of light quality on seed dormancy

The two field collections of seeds used (collected at the ends of 2000 and 2006 growing seasons) had a base (nonstratified) germination of *c.* 40% and were responsive to dark-stratification, with *c.* 80% of seeds germinating when transferred to germination conditions following 21 d stratification in the dark at 20°C (Fig. 2a). The similarity in germination response between the 2000 and 2006 collections enabled the results of repeated experiments to be combined. Stratification for 21 d under W, G_{filter}, G_{LED} or R_{LED} caused seeds to remain as dormant as nonstratified seeds (43% germination after 46 d), and B_{filter} and B_{LED} stratification resulted in only slightly higher germination than nonstratified seeds (Fig. 2). By contrast, stratification under far-red light (FR) or R_{filter} (which contained a high proportion of FR) caused significant loss of dormancy to *c.* 70% germination after 46 d, approaching the 80% germination of dark-stratified seeds (Fig. 2b).

Effect of light quality on dark-stratified (nondormant) seeds

The response of dark-stratified (and thus nondormant) seeds to subsequent light stratification varied depending upon the

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Table 1 Duration and fluence rate of light-emitting diode (LED) light pulses used to determine how much light is required to inhibit dark-induced dormancy release in *Lolium rigidum* seeds

Light quality	Total fluence (μmol m^{-2})	Fluence rate code	Fluence rate (μmol m^{-2} s^{-1})	Total time in light	Duration of each light pulse
Expt 1					
Blue	3.7×10^5	(i)	13.2	7.9 h	1.1 h
		(ii)	6.2	16.7 h	2.4 h
		(iii)	2.9	35.8 h	5.1 h
		(iv)	0.2	21 d	Constant light
Green	5.0×10^5	(i)	14.8	9.4 h	1.4 h
		(ii)	6.7	20.7 h	3.0 h
		(iii)	3.2	43.6 h	6.2 h
		(iv)	0.3	21 d	Constant light
Red	5.4×10^5	(i)	13.6	10.9 h	1.6 h
		(ii)	6.4	23.4 h	3.3 h
		(iii)	2.0	75.8 h	10.8 h
		(iv)	0.3	21 d	Constant light
Expt 2					
Blue	5.3×10^6	(i)	13.2	110.5 h	15.8 h
		(ii)	6.2	234.7 h	33.5 h
		(iii)	2.9	21 d	Constant light
Green	5.8×10^6	(i)	14.8	109 h	15.6 h
		(ii)	6.7	239.4 h	34.2 h
		(iii)	3.2	21 d	Constant light
Red	3.6×10^6	(i)	13.6	89.8 h	10.4 h
		(ii)	6.4	155.7 h	22.2 h
		(iii)	2.0	21 d	Constant light

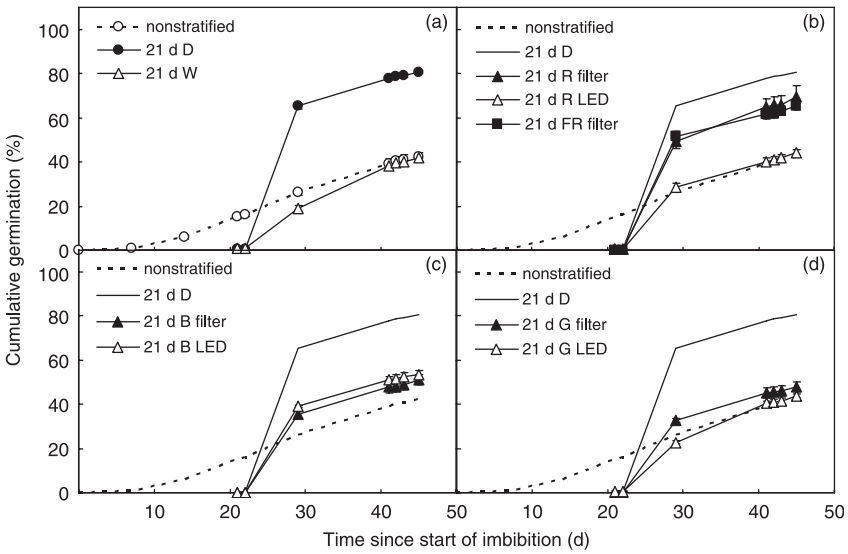

Fig. 2 Germination response of dormant seeds to stratification under different light qualities. Dormant seeds of *Lolium rigidum* were stratified for 21 d at 20°C under white (W) (a), red (R) or far-red (FR) (b), blue (B) (c) or green (G) (d) light and then placed in germination conditions (25 : 15°C, 12 h photoperiod) for a further 25 d. R, B and G light qualities were supplied by either filtering W light through Lee filters or by light-emitting diode (LED) arrays (spectra are shown in Fig. 1). For clarity, lines for the nonstratified and D-stratified results (a) are repeated in (b–d). Values are means ± 1 SE, n = 4; pooled LSD value is 6.3.

quality and regime of the light treatment. Dark-stratified seeds exposed to W in 12 h light : dark cycles (equivalent to standard germination conditions) germinated immediately, reaching 80% germination after the 21 d W and continuing on to a maximum of 90% by the end of the subsequent 24 d (Fig. 3b). Dark-stratified seeds exposed to R in 12 h light : dark cycles also germinated, reaching 60% during the R treatment and continuing to > 90% during subsequent incubation in standard germination conditions (Fig. 3e). Dark-stratified seeds that were exposed to all other conditions, including W or R delivered constantly, were unable to germinate until subsequently transferred to standard germination conditions (Fig. 3). Once under normal germination conditions, seeds which had been exposed to FR (constant or with a 12 h

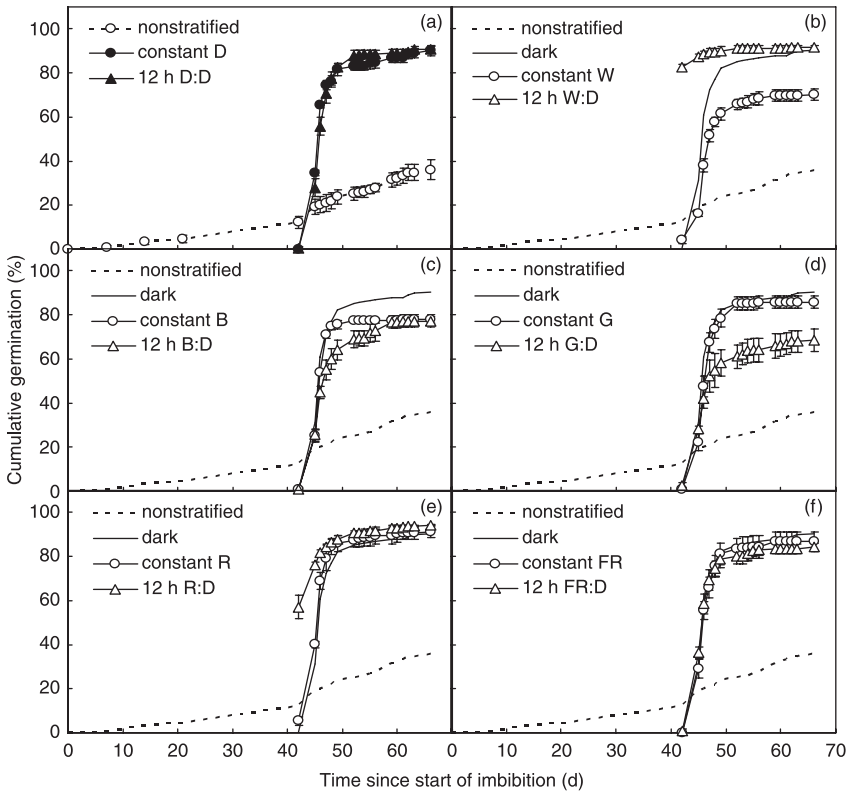

Fig. 3 Response of nondormant (dark-stratified) seeds to subsequent light stratification. During the first 21 d of imbibition, dormant seeds of *Lolium rigidum* were stratified in the dark at 20°C to promote dormancy release. For the next 21 d, the now nondormant seeds were transferred to stratify under either constant light at 20°C or 12 h light : dark cycles at 25 : 15°C, before finally being transferred to standard germination conditions (25 : 15°C, 12 h white : dark cycles) from 42 d. The second stratification phase was carried out in the dark (D) (a), or under white (W) (b), blue (B) (c), green (G) (d), red (R) (e) or far-red (FR) light (f). For clarity, lines for the nonstratified results (a) are repeated in (b) to (f), and germination of seeds following D stratification for 42 d in (a) were averaged and shown as a single trace in (b) to (f). All light qualities were provided by filtering W through Lee filters. Values are means ± 1 SE, n = 4; pooled LSD value is 7.9.

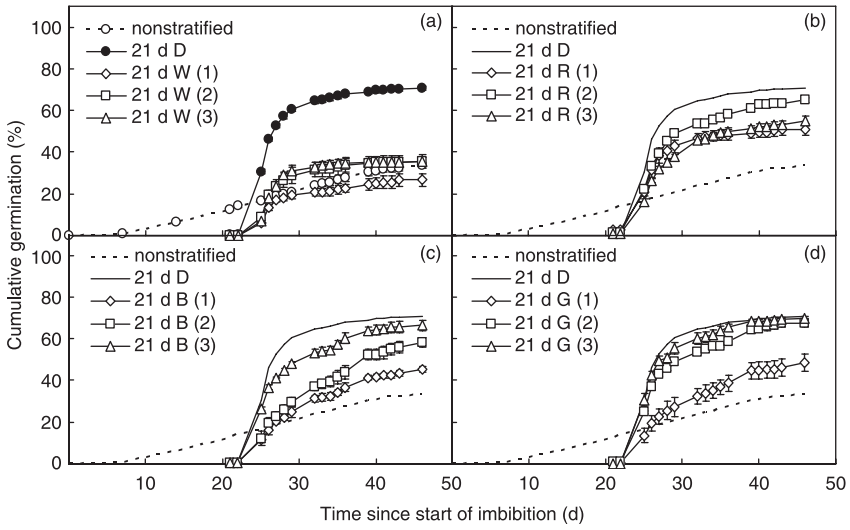

Fig. 4 Response of stratifying seeds to varying fluence rates. Dormant seeds of *Lolium rigidum* were stratified for 21 d at 20°C under white (W) (a), red (R) (b), blue (B) (c) or green (G) light (d) of three different fluence rates (average, in μmol m^{-2} s^{-1}) – 6.2 (1), 2.7 (2) and 0.3 (3) – and then transferred to germination conditions (25 : 15°C, 12 h photoperiod) for a further 24 d. W light was provided by halogen globes, and R, B and G by light-emitting diode (LED) arrays, all filtered through Lee 209 neutral density filters. For clarity, lines for the nonstratified and dark-stratified (D) results (a) are repeated in (b) to (d). Values are means ± 1 SE, n = 4; pooled LSD value is 7.2.

photoperiod), constant R or constant G, germinated to the same extent (*c.* 90% after 66 d) as those that remained in darkness for the 42 d (Fig. 3d–f). Dark-stratified seeds which had been under constant W, constant B, or 12 h photoperiod B or G retained some dormancy (70–80% germination after 66 d), but were still much less dormant than nonstratified seeds (Fig. 3b–d).

Effect of fluence rate on seed dormancy

Maintenance of dormancy by stratification of dormant seeds under W was independent of fluence rate, with original dormancy rates being retained at all fluences tested (0.3–6.2 μmol m^{-2} s^{-1}; Fig. 4a). Dormancy maintenance by stratification under B, G or R LED lights was fluence rate-dependent,

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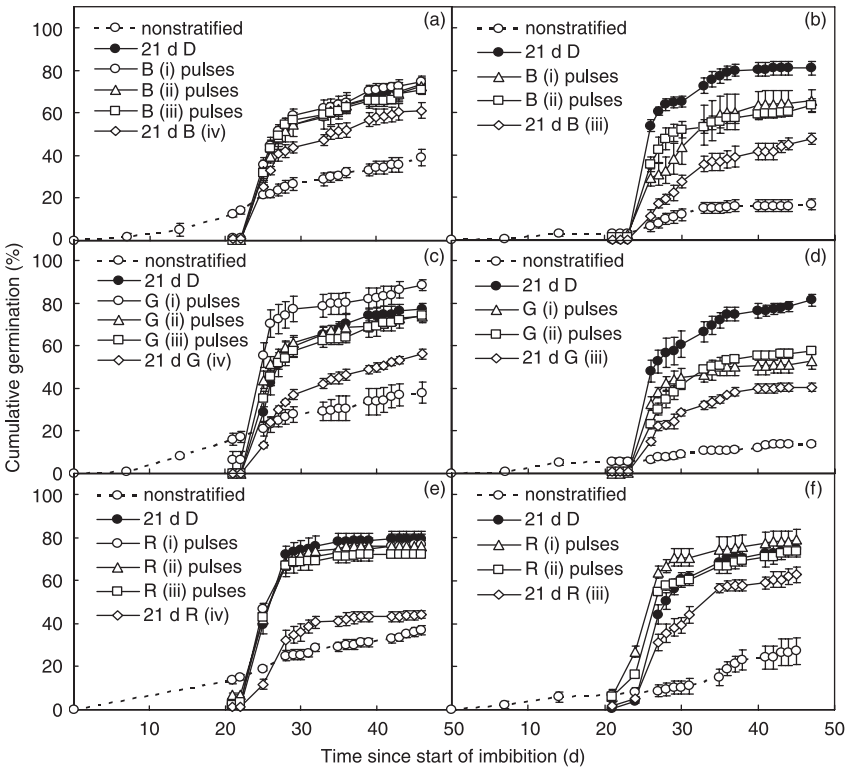

Fig. 5 Effect of light pulses on dormancy release in dark-stratifying seeds. Dormant seeds of *Lolium rigidum* were stratified for 21 d at 20°C under the light regimes specified in Table 1. Seeds received the lower total fluences of light (in μmol m^{-2}) – 3.7×10^5 of blue (B) (a), 5.0×10^5 of green (G) (c) and 5.4×10^5 of red (R) (e) – or the higher total fluences of 5.3×10^6 of B (b), 5.8×10^6 of G (d) and 3.6×10^6 of R (f). Following stratification, seeds were transferred to germination conditions (25 : 15°C, 12 h photoperiod). Nonstratified and dark-stratified (D) controls were performed at the same time. B, G and R light was provided by light-emitting diode (LED) arrays filtered through Lee 209 neutral density filters. Values are means ± 1 SE, $n = 4$; LSD values: (a) 9.3; (b) 10.3; (c) 9.2; (d) 7.6; (e) 8.6; (f) 11.7.

with fluence rate 1 (6.2 μmol m^{-2} s^{-1}) being the most effective at maintaining dormancy (*c.* 50% germination after 46 d) in all three cases (Fig. 4b–d). Indeed, differences in absolute dormancy release between experiments (cf. Fig. 2 vs Fig. 4) are linked not only to the different seed populations used but also to the different fluence rates involved, and so also point to fluence rate-dependent responses.

Effect of light pulses on dark-induced dormancy release

The first experiment, in which seeds were exposed to light pulses with the lower total fluence of *c.* 4.6×10^5 μmol m^{-2} (Table 1), showed that only the seeds exposed constantly to light (rate iv) retained any dormancy (40–60% germination). Interruption of dark-stratification with a pulse of light had no inhibitory effect on dormancy release, resulting in loss of dormancy equivalent to constant dark-stratification (*c.* 75% germination after 46 d) (Fig. 5a,c,e).

The second experiment, in which the light pulses were of longer duration and higher total fluence (*c.* 4.6×10^6 μmol m^{-2}; Table 1), resulted in greater maintenance of dormancy under B and G light, but not R. Seeds under constant B or G light at the lowest fluence rate (rate iii) remained more dormant (*c.* 45% germination after 47 d) than those under pulsed light treatments (60% germination), which in turn remained more dormant than seeds kept in constant darkness (80% germination) (Fig. 5b,d). By contrast, only the seeds stratified under constant, rather than pulsed, R (rate iii) retained some degree of dormancy (60% germination); as seen for the lower total fluence in the first experiment, seeds that experienced interruption of dark-stratification with R pulses lost dormancy to the same extent as seeds stratified in constant darkness (75% germination after 45 d) (Fig. 5f).

Discussion

Seed dormancy release in *L. rigidum* was strongly inhibited by monochromatic blue, green or red light. However, white light was more efficient at each of three low fluence rates tested than monochromatic red, green or blue light, suggesting that multiple photoreceptors play a role in the maintenance of dormancy in imbibed seeds.

Seeds stratified under constant blue and green light displayed a dormancy-maintenance response that was independent of the presence of far-red wavelengths, which were a dominant component of the filtered light but absent in the LED light. The fact that far-red light alone caused significant loss of dormancy suggests that the dormancy-maintenance response to blue and green light is blind to far-red, and is thus not mediated by the phytochromes. A comparison of the expected P_{fr} : P ratios of phytochrome (calculated as in Scopel *et al.*, 1991) under the various light qualities confirms that the dormancy-maintenance response is independent of the P_{fr} : P ratio. Seeds with a phytochrome P_{fr} : P of either 0.05 (far-red) or 0.69 (red filter) lost dormancy, whilst those with a P_{fr} : P of 0.05 (blue filter), 0.21 (green filter), 0.45 (blue LED), 0.57 (green LED),

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0.72 (white) or 0.87 (red LED) all maintained dormancy. This clearly demonstrates that the rate of dormancy following stratification under white, blue or green light is not related to the phytochrome P_{fr} : P.

Although there are very few data on the action or expression of cryptochromes or phototropins in seeds, we favour cryptochrome action as potentially explaining the response of *L. rigidum* seeds to blue light. Phototropin expression studies in rice (Jain *et al.*, 2007) and *Arabidopsis* (Jarillo *et al.*, 2001) have shown that these proteins are highly expressed in mature leaf tissue and almost absent in roots, stems and flowers (although neither study tested expression in seeds). Corresponding with this, the action of phototropin is strongly linked to optimization of photosynthetic efficiency (Christie, 2007). By contrast, cryptochromes are involved in many aspects of plant growth and development, and Poppe *et al.* (1998) demonstrated that cryptochromes can mediate blue light perception in *Arabidopsis* seeds. As they do not perceive red or far-red light (Ahmad *et al.*, 2002), the action of cryptochromes, rather than phytochromes, can also explain the nearly identical responses of the seeds to monochromatic blue light and a blue : far-red mixture.

If the dormancy-maintenance response of *L. rigidum* seeds is indeed mediated by cryptochromes under blue light, then their similar response to green light cannot be explained by the blue wavelengths emitted by the green LEDs (see Fig. 1 for the slight overlap between spectra), as cryptochrome action is blue : green-reversible. A comprehensive analysis of the mechanism of cryptochrome activation has demonstrated that the active, semiquinone form of the flavin chromophore is inactivated by green light (Bouly *et al.*, 2007), confirming previous observations that cryptochrome-mediated responses are reversed by green light (Talbott *et al.*, 2003). This suggests that in dormant *L. rigidum* seeds, the response to green light could be mediated by a noncryptochrome, nonphytochrome photoreceptor. A number of cryptochrome-independent responses to green light have been identified in plants (reviewed in Folta & Maruhnich, 2007 and outlined in the Introduction), although most of these act in the opposite direction to phytochrome- and cryptochrome-mediated responses. It is possible that seeds respond differently to green light than do other plant organs, as has been shown for the phytochrome high-irradiance response: continuous far-red light acts in the same direction as red light in inhibition of hypocotyl elongation, but in the opposite direction in seed germination (Casal & Sánchez, 1998).

The varied response of seed dormancy maintenance to red light indicates that phytochromes may play only a minor or redundant role in dormancy maintenance. Although stable forms of the normally light-labile phytochrome A have been found in dry seeds (Sineshchekov *et al.*, 2006) and under extremely high fluence rates of red light (Franklin *et al.*, 2007), it is likely that phytochrome A does not play a role in imbibed seeds subjected to the long-term, relatively low-fluence light treatments of this study. The strong dormancy-maintenance response observable under monochromatic red light, probably mediated by a stable phytochrome such as phytochrome B or phytochrome C, was greatly diluted by the presence of far-red light, even when the P_{fr} : P was as high as 0.69 (under the red filter). Under white light, therefore, it is expected that the contribution of phytochrome action to dormancy maintenance would be overshadowed by that of the blue and green light photoreceptors.

The experiments in which seeds were stratified mainly in the dark and exposed to regular blue, green or red light pulses demonstrated that in terms of dormancy maintenance, the amount of time spent in the light was more important than the fluence rate or total fluence of the light received. From the data in Fig. 5 and light conditions in Table 1, the seeds must be exposed to blue or green light pulses for at least 20% of the time spent in dark-stratification for dormancy release to be inhibited, with longer durations corresponding to greater dormancy maintenance, independent of the fluence rate. Pulses of red light were not effective in maintaining dormancy, regardless of their duration. This again points to a relatively insignificant role for phytochrome in ryegrass seed dormancy maintenance under natural conditions.

By contrast, germination of nondormant (dark-stratified) seeds appears to be mediated predominantly by phytochrome. Only seeds exposed to white : dark or red : dark cycles following dark-stratification were able to germinate, whilst those kept under constant light (any quality) or far-red, blue or green light : dark cycles remained quiescent until transferred to white light : dark cycles. This indicates that light in the 600–700 nm range, most efficiently perceived by type II phytochromes such as phytochrome B or phytochrome C (Fankhauser, 2001), is necessary for germination. In addition, it is possible that light : dark cycles have a greater stimulatory effect on germination than constant light, although temperature effects (25 : 15°C cycles under the light : dark regime, constant 20°C under the constant light regime) cannot be ruled out (Steadman, 2004).

Nondormant seeds exposed to constant white or blue light or blue : dark or green : dark cycles did not subsequently germinate to as high a percentage as the seeds in the other treatments, indicating the possibility that these environments may have re-induced dormancy in some of the seeds in the population. However, the fact that seeds still germinated to at least 60% under these conditions suggests that dormancy release in dark-stratified ryegrass seeds is a largely irreversible process (provided that the temperature and moisture conditions remain constant), and that the majority of seeds remain ready to germinate once favourable conditions occur.

In summary, this study has found that maintenance of dormancy in imbibed *L. rigidum* seeds is potentially mediated by the combined actions of blue (possibly cryptochrome) and green light photoreceptors, with phytochrome acting redundantly in this process. By contrast, germination of nondormant seeds is mediated primarily by phytochrome, as is

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the case in most light-requiring plant species. As there are no photoreceptor mutants of *L. rigidum*, it is not currently possible to supplement our physiological data with molecular biological evidence of which specific photoreceptor is involved in each response to monochromatic light. However, this situation should change in the future with expressed sequence tag and genome sequencing of *Lolium perenne* (Sawbridge *et al.*, 2003) and the development of efficient transformation techniques in this species (Bajaj *et al.*, 2006).

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