A summary of dormancy in annual ryegrass (*Lolium rigidum*) seeds: dry after-ripening versus imbibition in the dark

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Summary In the Australian Mediterranean type climate, annual ryegrass (*Lolium rigidum* Gaud.) seeds are set in spring, shed in early summer, after-ripen during the dry summer, and most germinate in autumn/winter. After-ripening rate is directly related to temperature, proceeding faster as temperature increases, and can be described in terms of thermal time accumulation.

Annual ryegrass seeds also possess an alternative mechanism for dormancy release, in which seeds respond to hydration in darkness. Dormant seeds do not germinate when hydrated in light or dark conditions, but they become sensitive to light during hydration in the dark for a few weeks. Germination is then permitted upon subsequent exposure to enough light.

This paper compares the two different mechanisms of dormancy release in annual ryegrass seeds.

Keywords Dormancy release, germination, thermal time, weed seed biology.

INTRODUCTION

Annual ryegrass (*Lolium rigidum* Gaud.) is the primary weed of southern cropping regions in Australia. A major reason for its persistence lies in the innate dormancy exhibited by this species, which can extend into the growing season. The majority of annual ryegrass seeds are dormant at maturity and gradually lose dormancy during the hot summer months, becoming ready to germinate with the first substantial rains of the growing season. However a proportion of the population remains dormant into the growing season and emerges within the crop, or remains in the seedbank until the following year.

To accurately predict weed emergence a basic understanding of the mechanisms and kinetics of dormancy release with respect to environmental parameters is required. This paper summarises the main points regarding two contrasting dormancy release mechanisms that exist in annual ryegrass seeds, in which dormancy can be lost through hot dry conditions or warm, wet, dark conditions.

MATERIALS AND METHODS

Mature annual ryegrass florets (hereafter called seeds) were collected at the Department of Agriculture research station at Wongan Hills, WA on 6th November 2000. The collection was made from an uncropped section within a wheat crop in a long-term cropping field. Seed moisture content at collection was 13.6 ± 0.3% on a fresh weight basis.

After-ripening conditions Seed moisture content was reduced to 7.8 ± 0.1% by equilibrating over 100% glycerol at 20°C for 10 d. Seeds were then packaged into foil bags to maintain moisture content and stored at constant 6, 20, and 35°C for up to 10 months. Samples were retrieved monthly for germination testing and moisture content measurement. Seed moisture content remained between 7 and 8% during the after-ripening period.

Germination conditions Seeds were germinated on 1% agar-water in 9 cm diameter circular petri dishes. Three replicate dishes of 50 seeds were germinated each time a sample was retrieved. Dishes were placed inside clear plastic bags in an incubator set at 12-hourly alternating 25/15°C, optimal for germination (Gramshaw 1972). Light (12 h per day during the warm phase) at 30-50 μmol m^{-2} s^{-1} was provided by two 40 Watt fluorescent white light tubes. Wrapping the dishes in aluminium foil provided darkness. The number of germinated seeds was counted 14 days after the start of the test. The criterion for germination was radicle protrusion to >5 mm. Inviability of soft, ungerminated seeds was confirmed by cutting through the seed. Samples of ungerminated firm seeds were assessed for viability by staining with 1% tetrazolium solution (International Seed Testing Association 1999). Seeds were sliced to expose the endosperm and incubated in 1% tetrazolium solution for 24 h in the dark at 30°C. Extent of pink staining was observed through a microscope, and complete staining of the embryo and aleurone was required to score a seed as viable.

RESULTS

Annual ryegrass seeds were after-ripened at three different constant temperatures and sampled monthly. Each month, germination was measured under standard conditions of 25/15°C with light during the warm temperature and darkness during the cool temperature.

100% of seeds were dormant at collection. The rate of dormancy release was essentially zero at 6°C, and was faster at warmer temperatures (Figure 1). In this population dormancy release was slow at all temperatures, taking 9 months at 35°C to reach 50% germination.

Germination was also tested at 25/15°C in the dark (Figure 2). No seeds germinated in the dark at collection. Germination in the dark improved with after-ripening time, and improvement was faster at warmer temperatures. Germination in the dark remained lower than germination in the light throughout the after-ripening period at all three temperatures.

When each monthly sample was taken, seeds were also imbibed on agar in the dark for 21 d before opening to the light for a further 14 d. At collection, when no seeds would germinate in the light (Figure 1) or dark (Figure 2), 21 d dark-imbibition allowed 40% of seeds to germinate when subsequently moved into the light (Figure 3). By comparison, it took 200 d of after-ripening at 35°C to achieve 40% germination in the light when dark-imbibition was not included.

Within three months of after-ripening at 35°C seeds became more responsive to dark-imbibition and around 90% of seeds were able to germinate following 21 d dark-imbibition. Seeds after-ripened at 20°C and 6°C improved in responsiveness to dark-imbibition in the same way as seeds after-ripened at 35°C (Figure 3). Notably, primary dormancy level remained unchanged during after-ripening at 6°C (Figs 1 and 2), but seeds still became able to respond to dark-imbibition (Figure 3).

DISCUSSION

In this paper comparison is made between germination of seeds imbibed in the light (Figure 1), germination of seeds imbibed in the dark (Figure 2), and germination in the light following imbibition in the dark (Figure 3).

As after-ripening proceeded, germination in the light and germination in the dark progressively improved, although at all stages more seeds were able to germinate in the light than in the dark. Improvement in germination was dependent on temperature, being faster at warmer temperatures (Figures 1 and 2), reflecting the fact that annual ryegrass seeds after-ripen on the soil surface under hot dry conditions. At 6°C, dormancy release did not occur and seeds remained almost 100% dormant, indicating that dormancy release proceeds at a much reduced rate when temperatures decline into the winter.

The present results confirm findings using seeds that matured in 1999 at the same site, and seeds collected from other sites. However, initial proportion of dormant seeds was lower, and dormancy release rate

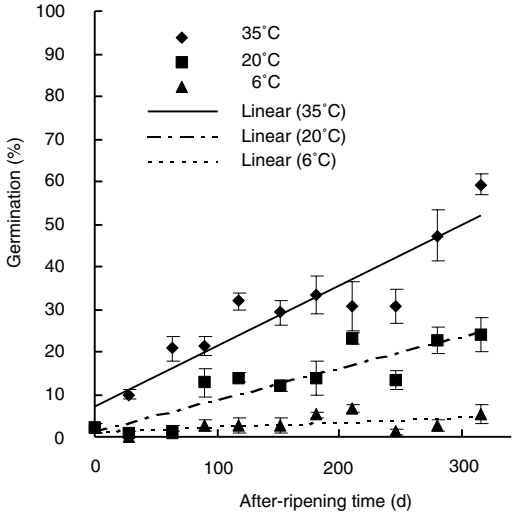

Figure 1. Change in germination in the light with after-ripening in annual ryegrass seeds collected from Wongan Hills, WA in November 2000. Seeds were stored at 6, 20, and 35°C for up to 10 months. Samples were taken at monthly intervals and germinated on agar at 25/15°C with light during the 12-hour warm period. Germination was counted 14 d after the start of the test. Bars are ± se of the mean.

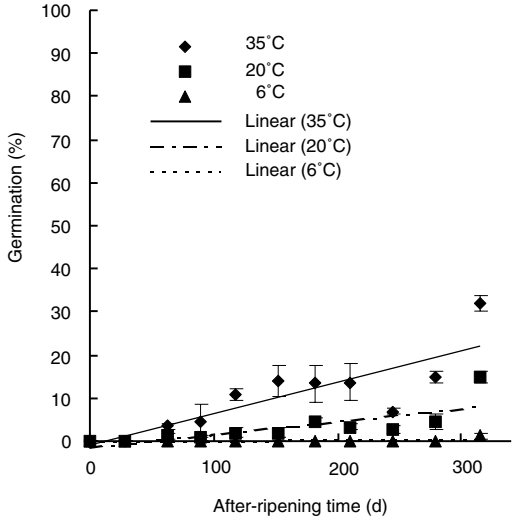

Figure 2. Change in germination in the dark with after-ripening in annual ryegrass seeds collected from Wongan Hills, WA in November 2000. Seeds were stored at 6, 20, and 35°C for up to 10 months. Samples were taken at monthly intervals and germinated on agar at 25/15°C in the dark. Germination was counted 14 d after the start of the test. Bars are ± se of the mean.

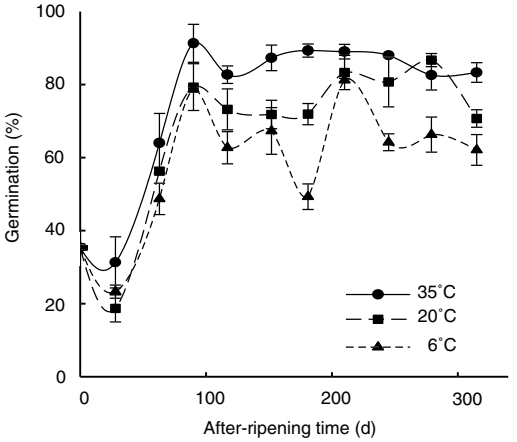

Figure 3. Change in responsiveness of annual ryegrass seeds to dark-imbibition for 21 d. Seeds were stored at 6, 20, and 35°C for up to 10 months. Samples were taken at monthly intervals and germinated on agar at 25/15°C. Plates were wrapped in foil to provide darkness for 21 d, and then unwrapped and left in 12-hourly light/dark. Symbols depict the sum of germination that occurred during the 21 d dark treatment and during the subsequent 14 d light/dark. Bars are ± se of the mean.

was slower in seeds collected in 2000 (Figure 1) than seeds in 1999 (Steadman *et al*. 2002).

Annual ryegrass seeds have the ability to circumvent the need for after-ripening. Seeds become able to germinate in the light if they are first allowed to imbibe in the dark for a few weeks. Seeds of the parasitic *Orobanche* spp. increase in sensitivity to a germination stimulant when they are imbibed in the dark for 7–21 days (Kebreab and Murdoch 1999), while *Sisymbrium officinale* (L.) Scop. require only 9 to 24 hours. In the case of *Orobanche*, the stimulant that seeds become more sensitive to is a chemical, strigol, derived from the host plant, while *S. officinale* becomes sensitive to simultaneous light and nitrate. The stimulant that annual ryegrass seeds develop sensitivity to is light.

While not all seeds in the population could respond to 21 d dark-imbibition directly after harvest, responsiveness improved quickly (Figure 3). Three months after the start of after-ripening, there was a dramatic difference in germination between seeds that received darkness for 21 d (Figure 3) and those that were placed straight into the light to germinate (Figure 1). The improvement in the ability of dark-imbibition to sensitise seeds to light was independent of temperature.

This method for stimulating germination of otherwise dormant seeds has potential for enabling testing (e.g. for herbicide resistance) of highly dormant populations in early summer. Every population tested to date (25) has been responsive to dark-imbibition (Steadman, unpub.).

The sensitisation to light by imbibition in the dark may be important in dormant annual ryegrass seed that becomes buried. Seeds will be sensitised to light within three weeks of becoming wet while buried, and any seeds that then move to the surface, perhaps through cultivation, will be able to germinate. Preliminary results suggest that seeds need to experience over 24 hours of light after dark-imbibition for germination to be stimulated, with a flash of light not being sufficient. Further research is required to identify the role that dark-imbibition may play and any potential for taking advantage of the response in weed control strategies.

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