Aspects of seed germination in the dune-building grass *Leymus arenarius*

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**SUMMARY**

The process of germination and the effects of several physical factors on the outcome of germination tests in *Leymus arenarius* seeds were examined. Harvest time was found to affect subsequent germination significantly; early harvested seeds had lower total germination than seeds harvested two weeks later in the autumn. Although the air-dry moisture content of seeds did not vary with their mass, smaller seeds imbibed water more quickly than larger ones. Stratification for 14 days at 5°C was a prerequisite for nearly complete germination but stratification did not affect the initial rate of germination. A brief exposure (1 min) to ambient laboratory light once a day did not reduce germination, despite the fact that light normally inhibits germination in this species; hence no special precautions are required for daily germination counts. Seed hulls (lemmas and paleas) did not impede final total germination, but de-hulled caryopses did germinate faster. These results are discussed in relation to the use of seeds in land reclamation programmes.

Key words: dormancy, lymegrass, reclamation, sand dunes, stratification.

**INTRODUCTION**

Seeds of *Leymus arenarius* (L.) Hochst. are sown on a large scale to stabilize drifting sands in Iceland and USA (Greipsson, 1993; Greipsson and Davy, 1994a; Wright, 1994). Germination of those seeds has sometimes been a problem in the field, mainly because they have had to be collected from wild stands, with consequent variation in quality i.e. in germinability and seed mass (Greipsson, 1991). Seeds of *L. arenarius* have been re-
ported to exhibit a strong dormancy (Sigurðbjörnsson, 1960; Bjarnason, 1982). Seeds are regarded dormant if they fail to germinate given appropriate conditions for germination. Seed dormancy can be caused by: (1) an immature embryo, (2) a seed coat impermeable to water or gases, (3) mechanical impedance that inhibits embryo development, (4) unfulfilled special requirements for light and temperature and (5) the presence of substances that can inhibit germination (Mayer and Poljakoff-Mayber, 1986). Such delayed germination has evolved in response to unpredictable and adverse environmental conditions but it can also be a penalty if good conditions prevail (Silvertown, 1988). Early and synchronised germination, however, is more advantageous when seeds are used in land reclamation projects.

Germination of seed may require certain external stimuli or physical conditions, in particular (1) imbibition of water, (2) a cold pre-treatment (stratification), (3) a suitable temperature environment, (4) a suitable light environment (quality or quantity) (Bewley and Black, 1978, 1982; Bryant, 1985; Bradbeer, 1988). Such prerequisites tend to be species-specific and need to be identified in order to ensure germination when required for reclamation work. Differences in requirement have also been established between populations (Seneca and Cooper, 1971; Greipsson and Davy, 1995) and seeds with different germination behaviour may even develop on the same plant (Greipsson and Davy, 1995). Appropriate experimental conditions for germination clearly should reflect environmental conditions of the microhabitat in the field and may act as phenological signals for germination. The optimal conditions for germination of *L. arenarius*, continuous darkness with diurnally alternating temperatures, were found to parallel field situations in volcanic sands in Iceland in spring (Greipsson and Davy, 1994b). However, other factors likely to affect germination remain to be addressed. In particular, in this paper we examine the effects of seed maturity, the influence of seed mass on water imbibition, the effect of stratification period, the consequences of brief exposure to light during germination, and whether the presence of hulls (lemmas and paleas) has any effect.

**METHODS AND MATERIALS**

*Effect of harvest time on germination behaviour*

Seeds were collected from the population at Krosssandur, on the south coast of Iceland, on three occasions in late summer of 1989: 21 August, 28 August and 8 September. Each sample of seeds was air-dried and then stored in a paper bag for no more than 6 months at room temperature until used for the germination test. For the test, seeds were allowed to imbibe water for 24 h, stratified at 5°C for 14 days and allowed to germinate at alternating temperature 12/12 h at 10/30°C in the dark, in Petri dishes (25 seeds each and replicated 4 times). Germination was monitored for 50 days. The data were examined using Life-table analysis (Scott et al., 1984; Greipsson and Davy, 1994b); this was carried out with the Survival analysis procedure of the SPSSX statistical package, VAX-VMS version (Nie, 1983). It makes use of both censored and uncensored data in calculating germination scores, it does not assume normally distributed data and it also accommodates heterogeneous variances. The method provides tests of whether groups differ significantly in terms of germination by both pairwise (without ranking) and overall comparisons using the D-statistic of Lee and Desu (1972), which is asymptotically distributed as chi-square with g–1 degrees of freedom, where g equals the number of groups. Apart from dormancy or insufficient time, failure to germinate may also be due to initial inviability or death during the experiment. Few caryopses showed signs of decay, suggesting that mortality was not an important factor. Therefore, the analysis was confined to surviving individuals; the total germination percent-
age presented (TG) refers to the number of caryopses that germinated as a proportion of all live individuals at the end of the experiment; the median germination time (Md) is the computed estimate of time for 50% germination of individuals remaining alive. Even when Md exceeds the period of observation and is indeterminate (where many seeds do not germinate but remain viable), significant differences in germination behaviour between treatments can be detected.

**Effect of seed mass on the rate of water imbibition**

Seeds from the population at Vatnsbærir were taken randomly from spikes and sorted into three groups on basis of mass. As before, seeds were air-dried and stored at room temperature in paper bags for up to 6 months. The moisture content of 20 air-dry seeds from each of the three mass-groups was obtained by weighing seeds individually before and after drying at 80°C for 48 h.

The rate of water imbibition was examined in another three samples of ten seeds, one from each of the mass-groups. Seeds were soaked in distilled-water and their mass were recorded at 2 h intervals for 48 h. Seeds were gently blotted on filter paper before weighing. The increase in seed mass was interpreted as an increase in seed moisture content.

**Effect of stratification on germination**

Germination with and without stratification was compared in seeds that had been collected from the population at Vatnsbærir, air-dried and then stored at room temperature in paper bags for up to 6 months. Stratification involved maintaining seeds at 5°C in wet sand for two weeks before the germination test. Control seeds that did not receive stratification were instead allowed to imbibe water for 24 h at room temperature, immediately prior to the germination test. Subsequently, germination was tested at a diurnally alternating (12/12 h) temperature of 10/30°C in the dark. Four replicates (Petri dishes) of 25 seeds were used for each treatment. The germination test continued for 30 days.

**Effect of a brief light exposure on germination**

Seeds that were nominally allowed to germinate in the dark did in fact receive a brief exposure of light each time germinated seed were counted. Consequently, the effects of a single brief exposure (1 min) per day to the ambient illumination (photon irradiance <100 µmol m⁻² s⁻¹) in the laboratory on germination were examined. Four treatments were used: (1) exposure to light (1 min day⁻¹) for four days, (2) exposure to light (1 min day⁻¹) for five days, (3) four days in continuous dark, (4) five days in continuous dark. Petri-dishes were wrapped in aluminium foil to avoid any exposure to light. Seeds were incubated at an alternating (12/12 h) temperature of 10/30°C in the dark. Four replicates (Petri dishes) of 25 seeds were used for each treatment. The germination test continued for 11 days.

**Effect of hulls on germination**

Seeds with and without hulls (i.e. lemma and palea) were allowed to germinate at alternating temperatures (12/12 h at 10/30°C) in the dark. Four replicates (Petri dishes) of 25 seeds with hulls were used and twelve replicates of 25 seeds without hulls were used. The germination test continued for 20 days.

**RESULTS**

**Effect of harvest time on germination behaviour**

The course of germination with time for seeds collected on the three dates is shown in Figure 1. The germination behaviour of seed from the third harvest (8 September) was significantly different from that of the two previous samples; the median germination time (Md) was much shorter and total germination (TG) was much higher (Table 1). Total germination in seed from the second harvest time (28 August) was even lower than in that from the first one (21 August).
**Table 1.** Effect of harvest time on germination behaviour. Seed from a population on the south coast of Iceland (Krosssandur) was collected at intervals of a few days. Seed mass is given for each harvest date and differences are compared using oneway ANOVA and subsequently Tukey’s multiple range test. Pairwise comparisons between harvest times were calculated. Means in each column followed by the same letter are not significantly different at P<0.05. Overall statistical comparison between harvest dates is given as Lee Desu’s D value. The median germination time is given (Md) and total germination is given (TG). Total number of seed used for each harvest time is given (n).

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Seed mass</th>
<th>Md</th>
<th>TG</th>
<th>Overall “D”</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 August</td>
<td>303</td>
<td>5.5±0.6 b</td>
<td>+50.0 b</td>
<td>55</td>
</tr>
<tr>
<td>28 August</td>
<td>290</td>
<td>10.5±0.1 a</td>
<td>+50.0 c</td>
<td>36 (P&lt;0.001)</td>
</tr>
<tr>
<td>8 September</td>
<td>270</td>
<td>11.2±0.2 a</td>
<td>8.6 a</td>
<td>98</td>
</tr>
</tbody>
</table>

Seed mass was nevertheless significantly higher for the second harvest time than the first one (Table 1). These results suggest that although harvest time can affect both seed mass and germination behaviour (Table 1), differences in germination behaviour are not entirely due to differences in seed mass.

**Effect of seed mass on the rate of water imbibition**

The moisture content of air-dry seeds was apparently independent of their mass, as no significant differences in moisture content were found between groups of different seed mass (Table 2). The progress of imbibition over 48 h for three seed-mass groups is shown in Figure 2. Smaller seeds consistently imbibed a higher percentage of water by mass than larger ones over the whole period. Water content at the end of the experiment was inversely related to seed mass.

**Effect of stratification on germination**

The progress of germination in seeds stratified for two weeks at 5°C and in unstratified controls is shown in Figure 3. The two treatments behaved similarly over the first 6 days, with approximately 50% of seeds germinating, but then germination levelled off more rapidly in the controls. After 30 days, germination approached 100% in the stratified seeds but had reached a plateau of only a little over 80% in the controls.

**Effect of a brief light exposure on germination**

The effect on total germination of a daily one-minute exposure to light is shown in Table 3. Seed given a brief daily exposure to light for 4 days had lower total germination than seed kept entirely in dark for 4 days but the difference was not significant. Another batch of seed given a brief daily exposure to light
Table 2. The moisture content of seed (mean±SD) in three mass-groups. Moisture was estimated by weighing seeds before and after drying them for 48 h at 80°C. Difference between moisture contents of seeds are compared using one-way ANOVA and subsequently Tukey’s multiple range test.

<table>
<thead>
<tr>
<th>Seed mass (mg)</th>
<th>Moisture content (F-value)</th>
<th>Seed mass (mg)</th>
<th>Moisture content (F-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3±0.2 a</td>
<td>278.84 (P&lt;0.001)</td>
<td>12.0±0.6 b</td>
<td>7.6±1.2 a (P&lt;0.013)</td>
</tr>
<tr>
<td>17.0±0.4 c</td>
<td>8.9±1.0 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exposure to light for 5 days had higher total germination than dark controls but, again, not significantly.

Effect of hulls on germination
Seeds without hulls had significantly earlier germination than seeds with hulls, reaching 50% germination 3 days earlier (Table 4). Germination of seeds with hulls began 6 days after incubation; at that time nearly all seeds without hulls had already germinated. Total germination (after 20 days) was virtually complete in both treatments and hence not significantly different between them.

Table 3. Effect of different exposures to light on germination. Seed were given a brief exposure (1 min) every day and germination compared with seeds kept in dark for 4 and 5 days. Total germination was compared using one-way ANOVA and subsequently Tukey’s multiple range test. Means in each column followed by the same letter are not significantly different at P<0.05. Significant differences are based on transformed data. Total germination (TG±SE) and the total number of seeds used for each treatment is given (n).

<table>
<thead>
<tr>
<th>Exposure to light</th>
<th>n</th>
<th>TG±SE</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every day</td>
<td>100</td>
<td>49±5.7  a</td>
<td>2.04</td>
</tr>
<tr>
<td>Dark for 4 days</td>
<td>100</td>
<td>61±6.6  a</td>
<td>(P&lt;0.2)</td>
</tr>
<tr>
<td>Every day</td>
<td>100</td>
<td>82±5.0  a</td>
<td>1.03</td>
</tr>
<tr>
<td>Dark for 5 days</td>
<td>100</td>
<td>77±6.0  a</td>
<td>(P&lt;0.035)</td>
</tr>
</tbody>
</table>

DISCUSSION
Seeds are harvested mechanically in Iceland each autumn and subsequently sown in a reclamation programme. Seeds do not remain on the spikes for long in the autumn because of strong gales. Therefore it is important to start the harvest before seeds are shed, but not so early that immature seeds are harvested. A later harvest time was found to give greater seed mass, more rapid germination and after 2 weeks’ stratification at 5°C were compared.

Figure 2. The progress of imbibition over time for three groups of seeds of different mean mass. Large: 16.6±1.1 mg; medium: 11.5±0.5 mg; small 7.2±0.5 mg.

Figure 3. Effect of low-temperature stratification on germination. Germination of seed without stratification and after 2 weeks’ stratification at 5°C were compared.
Table 4. Effect of seed hulls (lemmas and paleas) on germination. Seeds were allowed to germinate with or without hull. Pairwise comparisons between treatments was calculated. Means in each column followed by the same letter are not significantly different at P<0.05. Overall statistical comparison between populations is given as Lee Desu’s D value. The median germination time is given (Md). Total germination (TG) and total number of seeds used for each treatment is given (n).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>days</th>
<th>%</th>
<th>“D”</th>
</tr>
</thead>
<tbody>
<tr>
<td>With hulls</td>
<td>99</td>
<td>8.1</td>
<td>a</td>
<td>100</td>
</tr>
<tr>
<td>Without hulls</td>
<td>288</td>
<td>5.0</td>
<td>b</td>
<td>99</td>
</tr>
</tbody>
</table>

Imbibition of a certain amount of water is a prerequisite for germination (Bradford, 1990). The results reported here showed that smaller seed can imbibe water more quickly than larger ones even though their initial moisture content was not different. Smaller seeds may thus be favoured in particularly dry, well-drained sands. However, small seeds of *L. arenarius* were found to germinate more slowly after 24 h of imbibition than medium and larger sized ones (Greipsson and Davy, 1995). Clarke (1964) found that pre-soaking seeds of *L. arenarius* in water for up to 3 weeks increased germination. Seeds without glumes pre-soaked for 3 weeks showed 95% germination in 65 days, but seeds with glumes had only 70% germination (Clarke, 1964). Harris (1982) found that shaking seeds of *L. arenarius* for two weeks in water (at 20°C) before the germination test gave 53% germination after 25 days and 85% germination after 100 days at constant 20°C temperature in the dark. Clarke (1964) and Harris (1982) suggested that water-soluble inhibitors in the seed hull may need to be leached from seeds before germination. These findings were not corroborated in this study, unless the 24 h imbibition time is sufficient for leaching of water-soluble inhibitors.

Low-temperature stratification for 2 weeks was found to be necessary for complete germination. However, refractory populations have been found that need a longer time of stratification (Greipsson, 1991). Previous work had suggested that stratification is necessary for successful germination of seeds of *L. arenarius*. Sigurbjörnsson (1960) reported only 30% germination after moist seed had received 4°C and 22°C on alternate days for one week prior to three weeks germination test in the laboratory. Seed germinated best (close to 100%) after being kept in trays outside during November at Ithaca, New York, and subsequently brought into a greenhouse for 3 weeks (Sigurbjörnsson, 1960). In our
experiments, stratification was found to increase final germination but not to affect the initial rate of germination. Stratification may therefore not necessarily be a prerequisite for determination of $t_{50}$ (i.e. time until 50% of germinable seeds germinate) if seeds are incubated under a wide amplitude of alternating temperature. However, stratification should therefore be regarded as a prerequisite for germination tests aimed at determining total germination percentages. In contrast, Mayer et al. (1995) reported that stratification of 2 weeks for nondormant seeds of *Leymus cinereus* growing in western North America increased the rate of germination.

Light has previously been found to affect seed germination adversely; germinating seeds have poor prospects for surviving the harsh microclimate on the surface of sands and therefore light inhibition can prevent untimely germination (Greipsson and Davy, 1994b). Despite this, the results reported here suggest that a brief daily exposure to laboratory ambient illumination does not affect germination and therefore no precaution is needed when counting the seeds that have germinated.

Since hulls (lemma and palea) do not affect total germination it is not necessary to use cleaned caryopses in reclamation projects. Furthermore, in a field experiment it was found that seeds with hulls germinated significantly better than seeds without hulls, possibly because hulls protect seeds from drastic fluctuations in the moisture content of sands (Greipsson, unpublished data). This study corroborates the findings of Sveinsson and Björnsson (1994) that seeds with hulls germinate more slowly than seeds without hulls. However both these findings could be confounded by the fact that seed germination was defined as the first emergence of the radicle and as such it could not been seen so readily in seeds with hulls.

Seed germination and seedling establishment are regarded as the most critical stages in the survival of coastal dune grasses (Huiskes, 1977; Watkinson et al., 1979; Harris and Davy, 1987). Seed germination can be particularly difficult in sands because of their low water holding capacity, frequent desiccation and mobility. In the reclamation programme, seeds of lymegrass are usually coated with diatomaceous earth in order to prime them (Karssen et al., 1990; Gray, 1994), by aiding their water absorbing ability (Scott, 1975; Bradford, 1986). Application of gibberellins to seeds can also help break seed dormancy and aid seedling establishment (Karssen et al., 1989; Dunand, 1992). Hence, seeds may also be coated with the plant growth regulator Release (GA$_3$, 10%) in order to improve total germination (Greipsson, unpublished data). Early and synchronized germination is important so that seedlings can use the high rates of NPK fertilizer (400 kg ha$^{-1}$) that are applied before nutrients simply leach away. The results reported here should contribute to more objective procedures for the assessment of seed quality and the use of seed coatings, in order to improve the success of establishment in the field.

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