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# The Effect of Stratification and After-ripening Time on Seed Germination of Three Populations of *Arabidopsis lyrata* ssp. *lyrata* (Brassicaceae)

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**ABSTRACT** A good working knowledge of seed germination requirements is important for plant establishment for restoration and experimentation, particularly in wild plant species. Some seeds require a period of after-ripening and cold stratification before germination will occur. In this study we examined the effects of after-ripening and stratification time on seeds from three disjunct populations of *Arabidopsis lyrata* ssp. *lyrata*, from serpentine and limestone sand substrates of the Mid-Atlantic region of the United States. Differences among populations were evaluated through seed germination percentage and rate. Overall germination percentage and rate significantly varied by population, with seeds from the two serpentine populations having a lower germination percentage and rate than seeds from the limestone sand population. After-ripening time also significantly affected germination percentage and rate, with both measurements generally being the highest in 4 and 8 month-old seeds. Stratification did not generally alter germination percentage compared to the untreated control. However, stratification appeared to have the greatest effect on the germination rate of seeds that had after-ripened for 4 months, but the number of days of stratification required was not consistent across populations or across years. The variation observed among the populations of *A. lyrata* ssp. *lyrata* tested is a starting point for understanding how these differences developed and how they contribute to the success of each population in its native environment.

**INTRODUCTION** The establishment of wild plant populations, either as new crops or model organisms, requires a good working knowledge of their requirements for seed germination. Unreliable germination of wild plant populations can prove a hindrance in the introduction and successful establishment of new crops or hamper restoration efforts of wild populations (Aiken and Springer 1995, Jorge et al. 2007, Conversa and Elia 2008). If germination is unpredictable, an excess number of seeds need to be planted to ensure a large sample size for plant establishment and/or experimentation, with a subsequent waste of seed resources. This is particularly problematic when seeds are difficult to obtain, such as seeds collected from remote field locations or generated through hybridization (Pancholi et al. 1995). Knowledge of optimal storage conditions that maintain seeds in a predictable state of dormancy enhances the ability to conduct experiments year round (Cohn and Hughes 1981). Seeds of wild plant populations that are collected from a variety of environments will also often have different germination requirements from each other and those requirements will not necessarily be the same as other species within the same genus (Pancholi et al. 1995, Munir et al. 2001, Conversa and Elia 2008). Conditions that break dormancy and improve speed, unifor-

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mity and total germination can include after-ripening and cold stratification (Carrera et al. 2008, Conversa and Elia 2008).

*Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz (Brassicaceae) is a biennial/ perennial species (O'Kane and Al-Shehbaz, 1997) with a widespread but fragmented distribution in Europe, Asia and North America (O'Kane and Al-Shehbaz 1997, Mitchell-Olds 2001, Riihimaki and Savolainen 2004, Mable et al. 2005, Clauss et al. 2006, Mable and Adam 2007). Many of the areas where it grows are extreme environments that include granitic rock outcrops, serpentine soils, alluvial flood plains, and limestone sand (O'Kane and Al-Shebaz 1997, Mitchell-Olds 2001). There are three sub-species of *A. lyrata*. *Arabidopsis lyrata* ssp. *petraea* is distributed throughout Europe and boreal regions of Asia and North America (O'Kane and Al-Shehbaz 1997, Riihimaki and Savolainen, 2004). *Arabidopsis lyrata* ssp. *kamchatica* is found throughout Asia and boreal regions of North America (O'Kane and Al-Shehbaz 1997). *Arabidopsis lyrata* ssp. *lyrata* is found only in North America with a range on the East Coast as far north as Vermont and as far south as Georgia and as far west as the Great Lakes region (O'Kane and Al-Shehbaz 1997).

Much interest in *A. lyrata* has recently arisen due in part to its relationship to the model plant *Arabidopsis thaliana* (L.) (Kusaba et al. 2001). Although *A. thaliana* is an important model of plant growth and development, it has some limitations due to its relatively long history of growth under laboratory conditions. *Arabidopsis lyrata*, as a wild plant species with a history of exposure to stressful habitats, is an important model of molecular ecology and evolution in the Brassicaceae family (Koch et al. 1999, Mitchell-Olds 2001, Kärkkäinen et al. 2004). For example, Kärkkäinen et al. (2004) showed evidence of divergent selection for trichome production in *A. lyrata* ssp. *petraea*. A study on the evolution of and ecological consequences of changes in self-incompatibility in Great Lakes populations of *A. lyrata* ssp. *lyrata* was recently conducted (Mable et al. 2005, Mable and Adam 2007), which would be difficult to study in the predominantly self-pollinating *A. thaliana* (Mitchell-Olds 2001).

As *A. lyrata* is a wild plant species, there may be difficulty in having uniform germination to generate plants for experiments within the laboratory and greenhouse. This same difficulty in generating uniform germination from wild plant populations was observed in *A. thaliana* seeds collected from wild populations in Sweden (Nordberg and Bergelson 1999). In *A. thaliana*, it has been demonstrated that maternal environment has a significant effect on their progeny's germination and response to stratification (Munir et al. 2001). There is a substantial amount of variation in the maternal environments of *A. lyrata* populations, including granitic rock, limestone sand, and serpentine soils, which may affect their germination. Serpentine soils are particularly stressful due to the low calcium and high magnesium content of the soil and the presence of heavy metals such as nickel and chromium (Brady et al. 2005).

Germination in both *A. thaliana* and *A. lyrata* in their natural habitats is associated with cool temperatures and occurs in both the autumn and spring (Clauss and Koch 2006). Experiments involving the generation of *A. thaliana* and *A. lyrata* plants from seed usually utilize some exposure to cold stratification. High and uniform germination is stated as the reason for cold stratification treatments in *A. thaliana* (Vellanoweth 1997, Raghavan 2002); however, the exact amount of time used varies widely from one experiment to the next. For example, days of cold stratification used for *A. thaliana* include 2 (Hauser et al. 2001), 3 (Nordborg and Bergelson 1999, Donahue 2002, Schlesier et al. 2003), 4 (Raghavan 2002), 5 (Debeaujean et al. 2000), 21 (Munir et al. 2001), and 27 (Nordborg and Bergelson 1999). Nordborg and Bergelson (1999) found that in general a shorter stratification period (3 d compared to 27 d) improved overall germination in *A. thaliana*; however, they observed that there was no consistency across ecotypes. For *A. lyrata* the days of cold stratification that have been used to generate experimental plants are 0 (Mable et al. 2005), 2 (Hauser et al. 2001, Yogeeswaran and Nasralla 2006), 5 (Yogeeswaran and Nasralla 2006), and 21 (Riihimaki and Savolainen 2004). However, it is important to note that the stratification

times listed for *A. lyrata* were solely for the purpose of generating plants for experimental purposes, rather than testing germination of different populations. Seeds of *A. lyrata* ssp. *lyrata* are often collected in the wild and stored before being used for experiments so that the after-ripening time to which the seeds were exposed vary from one experiment to the next, which may alter germination percentage and speed of generating experimental plants. The purpose of this study was to determine the effect of stratification time and after-ripening time on seed germination of three disjunct populations of *A. lyrata* ssp. *lyrata*. We hypothesized that optimal germination would occur after 4 days of stratification in seeds that had after-ripened for 4 to 8 months but with the lowest germination in serpentine populations.

**MATERIALS AND METHODS** Seeds from three populations of *Arabidopsis lyrata* ssp. *lyrata* were used in this study. The collection sites include Pilot Preserve and Soldiers Delight Serpentine Barrens of Maryland and the Perry Preserve in the Dover Plains area of New York, which has a limestone sand substrate. Seeds were collected in late June and early July 2007 and 2008, with one week variation in collection time between years. Seeds from approximately 10% of the population were collected from all three locations by removing a single flowering stalk from plants that were at least 1 meter apart, with the seeds from each maternal plant being placed in a separate envelope. The number of plants sampled from Dover was 100 each year and 60 each year for Pilot and Soldiers Delight. The seeds collected in 2007 and 2008 were placed in dry storage in the dark at 23 ± 2°C for after-ripening and removed for germination testing after 1, 4, 8 and 11 months of after-ripening, with seeds from each year being used separately. The effect of stratification time on total germination (%) and speed and uniformity of germination (rate) were tested on seeds that were 1, 4, 8, or 11 months old (after-ripening time). The five stratification treatments were as follows: 0 days, 1 day, 2 days, 3 days, or 4 days exposure to 4°C in the dark. The stratification times were based on standard protocols for treating *A. thaliana* (Nordborg and Bergelson 1999, Hauser et al. 2001, Donahue 2002, Raghavan 2002, Schlesier et al. 2003), protocols for stratification treatment of wild relatives of *A. thaliana* (Yogeeswaran and Nasrallah 2006), and recommendations of standard treatment from other *A. lyrata* researchers (B. Roche pers. comm.). After 1, 4, 8, and 11 months of after-ripening, twenty seeds each from twenty two half-sib families were removed from their seed packets and pooled within each population for germination tests. The seeds were sterilized for 3 minutes in a 50% ethanol-Tween mixture and rinsed with 90% ethanol, which was allowed to evaporate before seeds were sown. Twenty seeds from a single population were sown on 10 cm Petri plates, which contained two filter paper disks saturated with 6 mL of distilled water. The plates were sealed with parafilm and placed in the dark in the cold room at 4°C for stratification treatment. At each after-ripening time for both years, every stratification time for each population was replicated four times.

The plates were arranged in the growth chamber in a completely randomized design (Conversa and Elia 2008). Seed sowing was staggered so that all stratification time (days) treatments of seeds from a single after-ripening time were placed in an EGC M12 series (Chagrin Falls, Ohio) growth chamber on the same day. The growth chamber was maintained at 20°C with a day/night cycle of 14/10 h, with a light intensity of 120 mol photons $m^{-2}$ $s^{-1}$. Two measures of germination were made: germination percent and germination rate, which is a measurement of uniformity and speed of germination. Germination was monitored daily for 14 days. A seed was considered germinated at visible radicle emergence. Germination rate (seeds $d^{-1}$) was calculated using the formula from Maguire (1962): $GR = (G_1/D_1 + G_2/D_2 + \ldots + G_f/D_f)$, where $G_1$ to $G_f$ are the number of seeds germinated on the first through final day of counting ($D_1$ to $D_f$). In 2008 a subset of seeds that did not germinate had their seed coats removed and were stained for viability with tetrazolium (Peters 2000). Seeds that had partial or complete color change were scored as viable, while seeds with no color change were scored as non-viable. Only undamaged seeds were used for viability staining; therefore, the number of seeds that were stained varied at each after-ripening time due to the

**Table 1. ANOVA table for germination percent of three *Arabidopsis lyrata* ssp. *lyrata* populations exposed to five different stratification treatments at four after-ripening times. Significant effects are in bold**

| | Germination (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2007 Seed | | | 2008 Seed | | |
| Source of Variation | *df* | F | *P* | *df* | F | *P* |
| Replication | 3 | 1.05 | 0.3696 | 3 | 0.58 | 0.6276 |
| Population | 2 | 181.77 | **<.0001** | 2 | 540.09 | **<.0001** |
| Stratification time (d) | 4 | 1.95 | 0.1044 | 4 | 2.47 | **0.0467** |
| After-ripening (months) | 3 | 62.73 | **<.0001** | 3 | 49.76 | **<.0001** |
| Population × stratification | 8 | 1.84 | 0.0715 | 8 | 3.64 | **0.0006** |
| After-ripening × stratification | 12 | 1.98 | **0.0284** | 12 | 3.40 | **0.0002** |
| Population × after-ripening | 6 | 3.8 | **0.0014** | 6 | 18.30 | **<.0001** |
| Population × stratification × after-ripening | 24 | 1.12 | 0.324 | 24 | 2.04 | **0.0046** |
| Error | 177 | | | 177 | | |

difficulty in removing the seed coat for staining without damaging the seed. Subsequently the raw data only could be presented without any statistical analysis.

Germination percentage and rate data were analyzed using the general linear model platform of JMP 8 (SAS Institute, Cary, North Carolina) with replication, population, stratification time (days), after-ripening time (months), population × stratification time, after-ripening × stratification time, population × after-ripening, and population × stratification time × after-ripening as the factors in the model. Comparisons of stratification treatments to the 0 day control, at each after-ripening time, were made through linear contrast (SAS Institute, Cary, North Carolina). The populations, at each after-ripening time point, and after-ripening times within each population were compared to each other using Tukey's HSD (SAS Institute, Cary, North Carolina). A *P*-value of ≤0.05 was considered significant throughout.

**RESULTS** Source population had a significant effect on both germination percentage and rate (Tables 1 and 2). Seeds from Perry Preserve consistently had the highest germination percentage and germination rate at all after-ripening times for both years of the study, while seeds from Soldiers Delight had consistently the lowest (Tables 3 and 4). Seed age (after-ripening time) also had a significant effect on germination percentage and germination rate, with a significant population × after-ripening interaction (Tables 1 and 2). The seeds from Perry Preserve had the highest germination percentage and germination rate of 4 and 8 month-old seeds in 2007 and 1 and 4 month-old seeds in 2008 (Tables 3 and 4). The seeds from Pilot and Soldiers Delight had the

**Table 2. ANOVA table for germination rate of three *Arabidopsis lyrata* ssp. *lyrata* populations exposed to five different stratification treatments at four after-ripening times. Significant effects are in bold**

| | Germination Rate | | | | | |
|---|---|---|---|---|---|---|
| | 2007 Seed | | | 2008 Seed | | |
| Source of Variation | *df* | F | *P* | *df* | F | *P* |
| Replication | 3 | 1.27 | 0.287 | 3 | 0.08 | 0.9709 |
| Population | 2 | 262.42 | **<.0001** | 2 | 625.71 | **<.0001** |
| Stratification time (d) | 4 | 3.66 | **0.0068** | 4 | 10.01 | **<.0001** |
| After-ripening (m) | 3 | 78.31 | **<.0001** | 3 | 72.81 | **<.0001** |
| Population × stratification | 8 | 2.5 | **0.0136** | 8 | 7.55 | **<.0001** |
| After-ripening × stratification | 12 | 3.27 | **0.0003** | 12 | 3.23 | **0.0003** |
| Population × after-ripening | 6 | 5.05 | **<.0001** | 6 | 41.14 | **<.0001** |
| Population × stratification × After-ripening | 24 | 1.30 | 0.1693 | 24 | 1.29 | 0.1778 |
| Error | 177 | | | 177 | | |

**Table 3. Mean ± standard error germination (%) of three *Arabidopsis lyrata* ssp. *lyrata* populations subjected to five stratification treatments at four after-ripening times. Populations within the same column and the same year with the same lower-case letter are not significantly different according to Tukey's HSD ($P \leq 0.05$). After-ripening times within the same population with the same upper-case letter are not significantly different according to Tukey's HSD ($P \leq 0.05$). Stratification treatments within a population and after-ripening time marked by an * were significantly different from the 0 day control according to Linear Contrast ($P \leq 0.05$)**

| | | Germination (%) | | | |
|---|---|---|---|---|---|
| | | 2007 Seed | | | |
| | | After-ripening (months) | | | |
| Population | Stratification Time (d) | 1 | 4 | 8 | 11 |
| Perry Preserve | 0 | 40.0 ± 7.4 | 75.0 ± 9.6 | 97.5 ± 1.44 | 70.0 ± 23.4 |
| | 1 | 56.2 ± 9.0 | 97.5 ± 1.4 | 95.0 ± 3.5 | 92.5 ± 3.2 |
| | 2 | 38.8 ± 5.2 | 75.0 ± 16.7 | 95.0 ± 2.0 | 87.5 ± 2.5 |
| | 3 | 23.8 ± 9.9 | 85.0 ± 5.4 | 92.5 ± 6.0 | 45.0 ± 20.7 |
| | 4 | 38.8 ± 6.6 | 93.8 ± 2.4 | 86.3 ± 4.7 | 62.5 ± 20.9 |
| | **Average** | **39.5 ± 7.6 aC** | **85.3 ± 7.1 aAB** | **93.2 ± 3.5 aA** | **71.5 ± 7.8 aBC** |
| Pilot | 0 | 1.3 ± 1.3 | 11.3 ± 2.4 | 22.5 ± 8.3 | 46.3 ± 7.7 |
| | 1 | 2.5 ± 1.4 | 40.0 ± 8.2* | 31.3 ± 3.2 | 37.5 ± 14.8 |
| | 2 | 5.0 ± 3.5 | 23.8 ± 7.2 | 48.8 ± 3.8* | 33.8 ± 9.4 |
| | 3 | 5.0 ± 2.0 | 13.8 ± 4.7 | 46.3 ± 1.3* | 27.5 ± 9.2 |
| | 4 | 5.0 ± 2.9 | 62.5 ± 4.3* | 45.0 ± 8.4* | 30.0 ± 3.5 |
| | **Average** | **3.8 ± 2.3 bC** | **30.2 ± 5.3 bB** | **38.8 ± 5.0 bAB** | **35.0 ± 4.1 bB** |
| Soldiers Delight | 0 | 1.3 ± 1.3 | 5.0 ± 2.4 | 28.8 ± 4.3 | 37.5 ± 1.4 |
| | 1 | 3.9 ± 3.9 | 5.0 ± 2.9 | 22.5 ± 9.2 | 16.3 ± 5.2 |
| | 2 | 0 ± 0 | 10.0 ± 3.5 | 33.8 ± 14.2 | 27.5 ± 13.9 |
| | 3 | 2.5 ± 1.4 | 10.0 ± 5.4 | 15.0 ± 2.9 | 11.3 ± 3.8 |
| | 4 | 0 ± 0 | 17.5 ± 4.8* | 16.3 ± 5.5 | 20.0 ± 6.1 |
| | **Average** | **1.5 ± 1.3 bC** | **8.5 ± 3.7 cB** | **23.2 ± 7.2 cA** | **22.5 ± 3.6 bAB** |
| | | 2008 Seed | | | |
| | | After-ripening (months) | | | |
| Population | Stratification Time (d) | 1 | 4 | 8 | 11 |
| Perry Preserve | 0 | 97.5 ± 2.5 | 96.3 ± 1.3 | 69.0 ± 9.8 | 60.0 ± 5.4 |
| | 1 | 97.5 ± 1.4 | 89.0 ± 1.0 | 73.0 ± 2.9 | 46.0 ± 12.6 |
| | 2 | 98.8 ± 1.3 | 98.8 ± 1.3 | 88.8 ± 3.2 | 61.3 ± 6.6 |
| | 3 | 100 ± 0 | 98.8 ± 1.3 | 76.3 ± 3.2 | 70.0 ± 10.6 |
| | 4 | 95.0 ± 5.4 | 93.8 ± 3.8 | 81.3 ± 5.9 | 75.0 ± 5.4 |
| | **Average** | **97.8 ± 1.2 aA** | **95.3 ± 1.2 aA** | **77.7 ± 2.7 aB** | **62.4 ± 4.1 aC** |
| Pilot | 0 | 25.0 ± 7.4 | 36.3 ± 4.3 | 37.5 ± 7.5 | 12.5 ± 7.8 |
| | 1 | 6.3 ± 4.7 * | 41.0 ± 5.5 | 32.5 ± 6.0 | 17.5 ± 4.8 |
| | 2 | 25.0 ± 12.4 | 2.5 ± 2.5* | 51.3 ± 9.0 | 13.8 ± 3.8 |
| | 3 | 46.3 ± 4.7 | 55.0 ± 8.4* | 32.5 ± 6.6 | 18.8 ± 1.3 |
| | 4 | 33.8 ± 13.3 | 18.8 ± 4.3 | 50.0 ± 9.1 | 28.8 ± 8.3 |
| | **Average** | **27.2 ± 4.8 bB** | **30.7 ± 4.7 cAB** | **40.8 ± 3.6 bA** | **18.2 ± 2.6 bB** |
| Soldiers Delight | 0 | 15.0 ± 3.5 | 65.0 ± 11.7 | 31.3 ± 4.3 | 25.0 ± 11.7 |
| | 1 | 7.5 ± 3.2 | 50.0 ± 3.5 | 32.5 ± 7.8 | 1.3 ± 1.3* |
| | 2 | 6.3 ± 3.2 | 53.8 ± 6.6 | 30.0 ± 4.1 | 6.3 ± 2.4 |
| | 3 | 11.3 ± 2.4 | 38.8 ± 6.3* | 26.3 ± 8.3 | 8.8 ± 5.5 |
| | 4 | 3.8 ± 2.4* | 26.3 ± 4.3* | 32.5 ± 10.3 | 8.8 ± 2.4 |
| | **Average** | **8.8 ± 1.5 cC** | **46.8 ± 4.1 bA** | **30.5 ± 3.0 cB** | **10.0 ± 3.0 cC** |

highest germination percentage and germination rate of 8 and 11 month-old seeds in 2007, and 4 and 8 month-old seeds in 2008 (Tables 3 and 4).

Stratification time also had a significant effect on germination percentage and rate (Tables 1 and 2); however, the effect was not observed at all seed ages and was also not

**Table 4. Mean ± standard error germination rate of three *Arabidopsis lyrata* ssp. *lyrata* populations subjected to five stratification treatments at four after-ripening times. Populations within the same column and the same year with the same lower-case letter are not significantly different according to Tukey's HSD ($P \leq 0.05$). After-ripening times within the same population with the same upper-case letter are not significantly different according to Tukey's HSD ($P \leq 0.05$). Stratification treatments within a population and after-ripening time marked by an * were significantly different from the 0 day control according to Linear Contrast ($P \leq 0.05$)**

| | | Germination Rate (seeds $d^{-1}$) | | | |
|---|---|---|---|---|---|
| | | 2007 Seed | | | |
| | | After-ripening (months) | | | |
| Population | Stratification Time (d) | 1 | 4 | 8 | 11 |
| Perry Preserve | 0 | 1.08 ± 0.022 | 2.87 ± 0.58 | 5.44 ± 0.38 | 3.37 ± 1.13 |
| | 1 | 1.87 ± 0.21* | 5.90 ± 0.14* | 5.44 ± 0.53 | 4.00 ± 0.26 |
| | 2 | 1.59 ± 0.28* | 3.80 ± 0.95 | 5.07 ± 0.54 | 4.52 ± 0.65 |
| | 3 | 0.79 ± 0.35 | 4.29 ± 0.17 | 5.00 ± 0.97 | 1.82 ± 0.78 |
| | 4 | 1.48 ± 0.20 | 6.00 ± 0.14 * | 5.39 ± 0.32 | 3.17 ± 1.06 |
| | **Average** | **1.36 ± 0.25 aC** | **4.57 ± 0.40 aA** | **5.27 ± 0.55 aA** | **3.38 ± 0.39 aB** |
| Pilot | 0 | 0.04 ± 0.04 | 0.29 ± 0.05 | 0.66 ± 0.23 | 1.63 ± 0.32 |
| | 1 | 0.05 ± 0.03 | 1.64 ± 0.47* | 1.34 ± 0.24 | 1.16 ± 0.48 |
| | 2 | 0.11 ± 0.09 | 0.81 ± 0.29 | 2.21 ± 0.19* | 1.05 ± 0.38 |
| | 3 | 0.17 ± 0.10 | 0.47 ± 0.17 | 1.90 ± 0.03* | 1.02 ± 0.34 |
| | 4 | 0.18 ± 0.11 | 3.11 ± 0.39* | 1.67 ± 0.27* | 1.07 ± 0.17 |
| | **Average** | **0.11 ± 0.07 bB** | **1.27 ± 0.28 bA** | **1.55 ± 0.19 bA** | **1.18 ± 0.15 bA** |
| Soldiers Delight | 0 | 0.02 ± 0.02 | 0.10 ± 0.04 | 1.01 ± 0.26 | 1.35 ± 0.10 |
| | 1 | 0.07 ± 0.07 | 0.10 ± 0.06 | 0.71 ± 0.33 | 0.47 ± 0.20 |
| | 2 | 0.0 ± 0.0 | 0.36 ± 0.11 | 1.36 ± 0.65 | 0.94 ± 0.46 |
| | 3 | 0.06 ± 0.04 | 0.32 ± 0.18 | 0.53 ± 0.12 | 0.29 ± 0.13 |
| | 4 | 0.0 ± 0.0 | 0.62 ± 0.17* | 0.41 ± 0.17 | 0.65 ± 0.21 |
| | **Average** | **0.03 ± 0.03 bC** | **0.30 ± 0.11 cB** | **0.81 ± 0.31 cAB** | **0.74 ± 0.13 bAB** |
| | | 2008 Seed | | | |
| | | After-ripening (months) | | | |
| Population | Stratification Time (d) | 1 | 4 | 8 | 11 |
| Perry Preserve | 0 | 4.62 ± 0.64 | 4.19 ± 0.05 | 2.97 ± 0.49 | 1.75 ± 0.11 |
| | 1 | 4.39 ± 0.40 | 4.58 ± 0.31 | 2.76 ± 0.32* | 0.96 ± 0.38* |
| | 2 | 5.59 ± 0.49 | 5.80 ± 0.39* | 3.98 ± 0.19 | 1.77 ± 0.18 |
| | 3 | 6.03 ± 0.77 | 6.25 ± 0.34* | 3.78 ± 0.42 | 2.01 ± 0.37 |
| | 4 | 6.91 ± 0.94 | 5.06 ± 0.23 | 4.41 ± 0.49* | 3.08 ± 0.36* |
| | **Average** | **5.51 ± 0.34 aA** | **5.18 ± 0.93 aA** | **3.58 ± 0.21 aB** | **1.91 ± 0.20 aC** |
| Pilot | 0 | 0.77 ± 0.33 | 0.90 ± 0.14 | 1.22 ± 0.25 | 0.26 ± 0.15 |
| | 1 | 0.18 ± 0.11* | 1.22 ± 0.16 | 1.01 ± 0.24 | 0.58 ± 0.22 |
| | 2 | 0.77 ± 0.47 | 0.05 ± 0.05* | 1.81 ± 0.25 | 0.40 ± 0.10 |
| | 3 | 1.93 ± 0.28* | 1.89 ± 0.30* | 1.25 ± 0.25 | 0.68 ± 0.09 |
| | 4 | 1.30 ± 0.57 | 0.54 ± 0.20 | 2.00 ± 0.25 | 0.96 ± 0.22* |
| | **Average** | **0.99 ± 0.20 bBC** | **0.92 ± 0.72 cBC** | **1.46 ± 0.14 bAB** | **0.39 ± 0.09 bC** |
| Soldiers Delight | 0 | 0.42 ± 0.12 | 2.10 ± 0.46 | 1.13 ± 0.08 | 0.58 ± 0.29 |
| | 1 | 0.14 ± 0.06 | 1.60 ± 0.08 | 0.96 ± 0.25 | 0.04 ± 0.04* |
| | 2 | 0.11 ± 0.05 | 2.41 ± 0.29 | 0.71 ± 0.05 | 0.10 ± 0.04* |
| | 3 | 0.33 ± 0.09 | 1.47 ± 0.22 | 0.78 ± 0.30 | 0.20 ± 0.15 |
| | 4 | 0.10 ± 0.06 | 0.73 ± 0.17* | 1.16 ± 0.45 | 0.35 ± 0.11 |
| | **Average** | **0.22 ± 0.04 cB** | **1.66 ± 0.77 bA** | **0.95 ± 0.12 cA** | **0.34 ± 0.08 bB** |

consistent between germination percentage and germination rate. In 2007, stratification alone had no significant effect on germination percentage (Table 1), while in 2008 stratification had a significant effect on 1 and 4 month-old seeds (Tables 1 and 3).

In both years, for germination percentage, there was a significant population × stratifi-

cation time interaction (Table 1). Stratification significantly affected germination percentage only for seeds from Pilot and Soldiers Delight. In 2008, 1 month-old seeds had reduced germination percentage compared to the 0 day control after 1 and 4 days of stratification for seeds from Pilot and Soldiers Delight, respectively (Table 3). In 2007, compared to the seeds from the 0 day control, 1 day of stratification significantly improved germination percentage in the seeds from Pilot and 4 days of stratification significantly improved germination percentage in 4 month-old seeds from both Pilot and Soldiers Delight. In 2008 stratification significantly improved germination percentage compared to the 0 day control only after 3 days in seeds from Pilot (Table 3). In 2007, stratification for 2 or more days significantly improved germination percentage compared to the 0 day control in seeds from Pilot only (Table 3).

Stratification time also had a significant effect on germination rate in both years (Table 2). In 2007 a significant effect was observed only in 4 month-old seeds, while in 2008 a significant stratification effect was seen at all seed ages (Table 4). The population × stratification time interaction was significant within both years (Table 2). This interaction was seen in 1 month-old seeds only in 2007, with improved germination rate in 1 and 2 day treated seeds from Perry Preserve. All 4 month-old seeds, in 2007, that had 4 days of stratification had a significantly higher germination rate than the 0 day control in all populations, with a similar significant effect seen in seeds treated for 1 day for seeds from only Perry Preserve and Pilot in 2007 (Table 4). Although stratification significantly improved germination rate in 2008 as well, a significant effect was only observed in seeds collected from Perry Preserve and Pilot (Table 4). The significant population × stratification time interaction was seen in 8 month-old seeds in 2007 only in seeds from Pilot, with at least 2 days or more of stratification having a significantly better germination rate compared to the control (Table 4). A significant population × stratification time interaction for 11 month-old seeds was seen only in 2008, with significantly better germination rate after 4 days of stratification in seeds from Perry Preserve and Pilot compared to the 0 day control, while 1 and 2 days of stratification appeared to have a reduced germination rate as they were significantly different from the 0 day control in seeds from Soldiers Delight (Table 4).

After-ripening time had a significant effect on both germination percentage and rate and had significant interactions with population and stratification time, but only a significant three-way interaction for germination percentage in 2008 (Tables 1 and 2). Germination percentage and rate in seeds from Perry Preserve was highest in 4 and 8 month-old seeds in 2007 and 1 and 4 month-old seeds in 2008 (Tables 2 and 4). In seeds from Pilot both germination percentage and rate were highest in 4 and 8 month-old seeds in both 2007 and 2008 (Tables 3 and 4). Germination percentage and rate were highest in seeds from Soldiers Delight in 8 and 11 month-old seeds in 2007 and 4 and 8 month-old seeds in 2008 (Tables 3 and 4). The after-ripening × stratification time interaction had the greatest effect on 4 month-old seeds (Tables 3 and 4).

The total number of seeds that germinated at each after-ripening time was over 50% for seeds from Perry Preserve and under 50% for seeds from Pilot and Soldiers Delight (Table 5). The lowest number of germinated seeds from Pilot and Soldiers Delight were at the 1 and 11 month after-ripening times (Table 5). The percent confirmed non-viable seeds from the subset of seeds that did not germinate from these two populations was higher in the 11 month-old seeds; although this was not tested statistically (Table 5).

**DISCUSSION** *Arabidopsis lyrata* is increasingly a subject of interest for those wishing to study a wild plant relative of the model plant *A. thaliana* (Koch et al. 1999, Kusaba et al. 2001, Kärkkäinen et al. 2004). *Arabidopsis lyrata* ssp. *lyrata* in particular is being used to study the evolution of mating systems (Mable et al. 2005, Mable and Adam 2007) and local adaptation to different soil types of the Eastern United States (Turner et al. 2008). Having high and uniform germination is important, particularly when growing plants derived from seed of wild populations in the laboratory or the greenhouse (Cohn and Hughes 1981, Pancholi et al. 1995, Conversa

**Table 5. Total seed germination and results of viability staining of non-germinated seeds collected from three *Arabidopsis lyrata* ssp. *lyrata* populations in 2008**

| Population | After-ripening (months) | Seeds Germinated | | Staining of Non-germinated Seeds | | |
|---|---|---|---|---|---|---|
| | | Yes | No | Viable | Non-viable | Unknown |
| Perry Preserve | 1 | 391 | 9 | 5 | 3 | 1 |
| | 4 | 382 | 18 | 3 | 1 | 0 |
| | 8 | 314 | 86 | 3 | 7 | 0 |
| | 11 | 239 | 161 | 11 | 6 | 3 |
| Pilot | 1 | 109 | 291 | 51 | 20 | 30 |
| | 4 | 122 | 278 | 7 | 3 | 0 |
| | 8 | 163 | 237 | 9 | 1 | 0 |
| | 11 | 73 | 327 | 13 | 8 | 0 |
| Soldiers Delight | 1 | 35 | 365 | 33 | 15 | 12 |
| | 4 | 187 | 213 | 4 | 0 | 0 |
| | 8 | 122 | 278 | 5 | 0 | 7 |
| | 11 | 40 | 360 | 9 | 10 | 1 |

and Elia 2008). High and uniform germination is especially important when dealing with seeds from different populations where the maternal plants are exposed to very different environments, which has been shown in *A. thaliana* to affect their seeds response to stratification (Munir et al. 2001). In *A. thaliana*, it is conventional to treat the seeds with a cold stratification period before germination (Vellanoweth 1997, Munir et al. 2001, Donahue 2002, Schleiser et al. 2003). For both *A. thaliana* and *A. lyrata* (including all three sub-species) there is no consistent procedure used to generate experimental plants, and none of the procedures described for *A. lyrata* were specifically testing germination and did not take into account differences among populations (Hauser et al. 2001, Munir et al. 2001, Riihimaki and Savolainen 2004, Mable et al. 2005). Initially we were attempting to discover the best stratification and after-ripening times to ensure optimal seed germination of the three populations, but instead we discovered interesting differences in the depth of dormancy of these three populations.

The seeds from Perry Preserve consistently had the highest germination percent and rate at every after-ripening time and under each stratification treatment (Tables 3 and 4). The dormancy of seeds from Perry Preserve seemed much more shallow compared to the seeds from Pilot and Soldiers Delight. Germination percentage of seeds from Perry Preserve was consistently above 70% at almost all after-ripening times and under each stratification treatment, while germination percentage for seeds from Pilot and Soldiers Delight was rarely above 50% (Table 3). Despite these differences in germination the seed viability among populations appears to be quite similar (Table 5), with an apparent decline in seed viability in the 11 month-old seeds that may be related to a decline in germination percent and rate. However, decline in seed quality is not the only explanation for low germination of *A. lyrata* ssp. *lyrata* seeds, particularly from the serpentine populations (Pilot and Soldiers Delight), which rarely had more than 50% of their seeds that germinated (Table 3). For these populations, the seeds may need to be exposed to additional treatments (perhaps a longer cold stratification period or alternating periods of warm and cold temperatures) before high uniform germination will occur (Baskin and Baskin 2004).

Our data point to a role for stratification in seed germination in *A. lyrata* ssp. *lyrata*. Stratification significantly affected germination rate more than overall germination percentage, but the amount of stratification needed was not consistent across populations (Table 4). There was a positive effect of stratification on germination rate, which was most evident in the 4 month-old seeds (Table 4). For the seeds from Perry Preserve and Pilot some of the stratification treatments had significantly higher germination rates than the 0 day control (Table 4). On the other hand, there were rarely significant differences in germination rate between the stratification treatments and the 0 day control in seeds from Soldiers Delight (Table 3).

The variation observed among these three populations, particularly between the seeds from Perry Preserve and the seeds from the two serpentine populations, for overall germination percentage and germination rate is not unexpected since they come from such different environments. The maternal plants for the seeds from Perry Preserve grow in limestone sand, while the maternal plants of the seeds from Soldiers Delight and Pilot grow in serpentine soil. Serpentine soils are particularly stressful due to the low calcium and high magnesium content of the soil and the presence of heavy metals such as nickel and chromium (Brady et al. 2005). The reduced germination percentage and rate seen in both serpentine populations indicates deeper dormancy than is found in seeds from Perry Preserve. The highest germination percentage and rate in seeds from Pilot and Soldiers Delight occurred after a longer after-ripening time than that observed for the highest germination percentage and rate in the seeds from Perry Preserve (Tables 3 and 4). In addition, the only significant increases in germination percentage in response to stratification occurred in the two serpentine populations (Table 3). We speculate that this deeper seed dormancy may increase success at later life stages in the highly stressful serpentine environment by ensuring that germination occurs under the most favorable conditions possible.

Rather unexpected were the differences observed between the seeds from the two serpentine populations. Stratification seemed to have the greatest effect on the seeds of the Pilot population, with a trend of stratification improving both germination percentage and rate (Tables 3 and 4). Although both Pilot and Soldiers Delight are part of the State Line serpentine barrens of Maryland and Pennsylvania there are significant differences between the two sites with half as much bare rock and soil at Soldiers Delight compared to Pilot (Tyndall and Farr 1990, Tyndall 1992). The growing season is about a month longer at Soldiers Delight than at Pilot (Tyndall 1992). However, the soils at Soldiers Delight are nutritionally poorer than those at Pilot, with lower levels of potassium, calcium, and magnesium, and a slightly lower pH, while the soils at Pilot have a higher concentration of nickel and chromium than the soils at Soldiers Delight (Hart 1980, Panaccione et al. 2001). Although plants from both Pilot and Soldiers Delight deal with many similar environmental stressors, the unique stresses encountered by each population may account for differences in seed germination between these two populations.

There are indications that both after-ripening time and stratification do play a role in breaking dormancy in *Arabidopsis lyrata* ssp. *lyrata* (Table 4). However, since germination was not consistently above 90% for all populations, longer periods of stratification and/or other currently unknown conditions are probably needed to break dormancy. Our data can act as a starting point to gain a more detailed understanding of seed dormancy in each population and how these differences developed in response to the population's environment, in addition to understanding how these germination differences contribute to the success of each population in its native environment.

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