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GERMINATION OF *Silene regia* SEEDS FROM FOUR SITES IN LAWRENCE COUNTY, ILLINOIS, FOLLOWING SCARIFICATION OR STRATIFICATION

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ABSTRACT: *Silene regia* Sims is an endangered prairie forb in Illinois where small isolated colonies are scattered. In Lawrence County, two sites (Allison Prairie and Chauncey Marsh) have fewer plants (6—23) than two other sites (County Road and Cemetery) with 26—45 plants. Information on seed germination in these isolated colonies is needed. Our goal was to evaluate seed germination of *S. regia* from colonies in Lawrence County, Illinois. *S. regia* fruits were collected from these four sites on August 9 and 19, 1999. Seeds were scarified by cutting the seed coat, or they were stratified at 2 C for 12 or 15 weeks. Seeds from Chauncey Marsh weighed less than those from other sites. With the exception of seeds from Chauncey Marsh, scarification increased germination within each site. When significant germination differences occurred due to site, they were apparent on stratified seed, where frequently Allison Prairie was highest and Chauncey Marsh was lowest. Germination differences between stratified and control seeds were inconsistent, although stratified seeds had up to 67% higher germination than control seeds when significant differences occurred. These increases in seed germination were most evident in seeds collected on August 9th and stratified for 12 weeks. Seed that was neither scarified nor stratified germinated after storage, indicating that scarification and stratification are not absolute germination requirements with after-ripened seeds. Seed germination at different sites did not correspond directly with population sizes, and multiple mechanisms were present for breaking seed dormancy in *S. regia*.

INTRODUCTION

Silene regia Sims (commonly known as royal catchfly) is an endangered prairie forb found sparingly in mesic prairies and oak savannas from southeastern Kansas to northeastern Illinois (Ladd 1995). Menges (1995) cites lack of fire and decreased pollinator visitation (ruby-throated hummingbirds in particular) as reasons for the diminished success of *S. regia*. More generally, habitat fragmentation and a severe decline in prairie habitats throughout the Midwestern states also have contributed to the endangered status of this species. According to Menges (1995), fire and soil disturbance have a positive effect on seed germination since seeds require light to germinate. Menges (1991) also indicated that inbreeding due to small population sizes of *S. regia*, has a negative effect on seed germination. Western populations of *S. regia* were more genetically diverse than eastern populations based on the Shannon-Weaver Index (Dolan 1994). Unlike the western populations of this species, genetic variation of *S. regia* was correlated positively with population size in the east (Dolan 1994).

Seed dormancy is reported in seeds of *S. regia*. Seeds of *S. regia* did not after-ripen during the summer (Baskin and Baskin 1988), but seed dormancy of *S. regia* was overcome by cold stratification (Baskin and Baskin 1988; Menges 1991, Menges 1995, Baskin and Baskin 1998). Studies focused on whether or not mechanical scarification

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was required for germination of *S. regia* were not found, although seeds of *Saponaria officinalis* L. (bouncingbet), also in Caryophyllaceae responded to scarification. Lubke and Cavers (1969) found that 100% of *S. officinalis* seeds germinated when scarified by nicking the seed coat with a razor. Moreover, scarification by shaking seeds for two days with limestone gravel and water from the Thames River in Canada, where the seeds were collected, yielded significantly higher germination of *S. officinalis* than no scarification, one day of scarification, or 3 to 5 days of scarification (Lubke and Cavers 1969). *S. regia* and *S. officinalis* also share a physical resemblance in the vegetative portions of their shoots, both having an opposite leaf arrangement and lanceolate leaves with entire margins. However, their success in the Midwest is radically different, with the former being endangered and the latter being an invasive species originating from Europe.

S. regia originally was reported in eleven counties of Illinois, although currently it only remains in four (Herkert and Ebinger 2002). In counties where it remains, colonies are small and fragmented. At four sites where it is still found in Lawrence County, Illinois, population sizes (number of individual plants) in 1997, 1998 and 1999 respectively, were 6, 11 and 13 for Allison Prairie; 35, 45 and 32 for County Road; 26, 30 and 38 for Cemetery; and 12, 23 and 11 for Chauncey Marsh (B. Edgin, personal observations). For Allison Prairie, multiple stems were present on each plant (Edgin et al. 2003). Allison Prairie and Chauncey Marsh plants are the remnants of 25 plants that were introduced to each site in October 1993. The transplants had been grown from seed collected from the Cemetery the previous year. Plants at the County Road and the Cemetery are naturally occurring. It is unknown whether these small population sizes have resulted in inbreeding and reduced seed germination as reported by Menges (1991) for other populations. The goal of this study was to evaluate the seed germination of *S. regia* from isolated colonies in Lawrence County, Illinois, and to compare how scarification and stratification affect their germination.

MATERIALS AND METHODS

Silene regia Sims fruits containing seeds were collected at four sites in Lawrence County, Illinois. These sites are within 32 kilometers of each other and have been labeled as Allison Prairie, County Road, Cemetery and Chauncey Marsh. Dried fruits were selected randomly from different plants with less than 10% of available fruits on each plant being removed. *Saponaria officinalis* L. seeds also were collected from the Cemetery site for a comparison in scarification studies. Fruits for *S. regia* were collected on August 9 and August 19, 1999, whereas those for *S. officinalis* were collected only on August 19, 1999. Seeds were removed from fruits by hand. Average seed masses were determined using three replications of 50 seeds each. Until the summer of 2000, seeds were stored at room temperature; and then they were moved to a seed storage chamber (4 C, 50% relative humidity).

Scarification studies

In fall 2000, 60 seeds of *Silene regia* from each of the four sites were randomly chosen, as well as 60 seeds of *Saponaria officinalis* from the August 19, 1999 collection. Thirty of the 60 seeds were scarified using a razor blade to break the seed coat. All seeds were dusted with thiram (tetramethylthiuram disulfide, 50% active ingredient) to decrease fungal contamination that might affect germination. Seeds were segregated by species, site, and scarification treatment. Each treatment of 30 seeds was divided into groups of ten for three replications. Low seed numbers were used due to limited seed availability. Seeds were placed into a 90 x 15 mm polystyrene Petri dish containing 5 ml of distilled water and two Whatman #1 filter paper disks. Petri dishes were sealed with Parafilm to maintain moisture within the dish. Dishes were placed into three 41.2 x 28.5 x 17.5 cm clear Rubbermaid® tubs with each tub containing a separate replication. Tubs were placed into a growth chamber at 25 C. The light intensity was 268 μmol m^{-2} s^{-1} for 14 h daily. Germinated seeds and moldy seeds were counted daily for 16 days with germination defined as the time when the radicle could be seen emerging from the seed. No further tests were done on ungerminated seeds.

Stratification studies

Seeds of *Silene regia* from both collection dates were stratified by placing seeds in moist paper towels within plastic bags and storing at 2 C. Stratification began on two different dates, October 19, 1999, and November 19, 1999. After 12 and 15 weeks of stratification, seeds from each site and collection date were removed. Thus, four stratification treatments were used: started in October for 12 weeks, started in October for 15 weeks, started in November for 12 weeks, and started in November for 15 weeks. Control seeds were stored in glass jars at room temperature (23 C) during the stratification of the other seeds. Five seeds per dish were dusted with thiram and then placed into each of three 90 x 15 mm glass Petri dishes with two disks of Whatman #1 filter paper and 5 ml of distilled water. Low seed numbers were used due to limited seed availability. Petri dishes were placed into 41.2 x 28.5 x 17.5 cm clear Rubbermaid® tubs in a seed germinator at 25 C with an average light intensity of 46 μmol m^{-2} s^{-1} for 16 h daily. Germinated seeds and moldy seeds were counted daily for 16 days with germination defined as the time when the radicle could be seen emerging from the seed. No further tests were done on ungerminated seeds.

Statistical analyses

The statistics program, Costat, was used to analyze the data by analysis of variance with a randomized complete block design, followed by mean separations using Duncan's multiple range test at the 5% level. Means and standard deviations also were calculated.

RESULTS

Seed characteristics

Table 1 shows the average masses of 50 *Silene regia* seeds harvested on August 9 and August 19, 1999. Seeds collected at Allison Prairie and County Road on August 9 were significantly heavier than those collected at the Cemetery and Chauncey Marsh on the same date. Seeds collected on August 19 from the County Road were significantly heavier than those from any other collection site. By comparison, the average mass of 50 *Saponaria officinalis* seeds was 77 ± 4 mg.

Seed coats of *Silene regia* seeds collected from all sites on August 9 were tan and dark brown, excluding those seeds from the Cemetery site, which were only tan. Seeds collected from Allison Prairie on August 19 were tan and maroon, while those collected from the Cemetery were gray and dark brown. The rest of the seeds collected on August 19 were tan and dark brown. Thus differences in seed color were present.

Scarification

Table 2 shows scarification effects. A significant scarification effect for *Silene regia* was noted, with higher germination in scarified seeds than non-scarified seeds within each site, excluding Chauncey Marsh. *Saponaria officinalis* also demonstrated a significant scarification effect with 100% germination when scarified and no germination when not scarified. All of the scarified *S. regia* seed had less germination than that of the *S. officinalis*. Also, non-scarified *S. officinalis* seeds had the lowest germination of all the treatments (0%).

Table 1. Masses (mg) of 50 *Silene regia* seeds from four sites in Lawrence County, Illinois, on two harvest dates.

Site	August 9, 1999	August 19, 1999
Allison Prairie	41 ± 2 a[1]	36 ± 7 bc
County Road	46 ± 2 a	60 ± 5 a
Cemetery	34 ± 4 b	45 ± 3 b
Chauncey Marsh	31 ± 3 b	34 ± 6 c

[1] Mean ± standard deviation. Means followed by different letters within a column are significantly different (Duncan's multiple range test, 5% level).

Table 2. Scarification effects on germination percentages of *Silene regia* and *Saponaria officinalis* seeds collected from different sites in Lawrence County, Illinois.

	S. regia		*S. officinalis*	
Site	scarified	non-scarified	scarified	non-scarified
Allison Prairie	80 ± 20 ab[1,2]	23 ± 15 b		
County Road	83 ± 15 a	23 ± 25 b		
Cemetery	80 ± 17 ab	70 ± 17 a		
Chauncey Marsh	50 ± 10 b	30 ± 10 b	100 ± 0	0 ± 0

[1] Mean ± standard deviation. Means followed by different letters within a column are significantly different (Duncan's multiple range test, 5% level).

[2] All means within a site (scarified vs. non-scarified) were significantly different with the exception of the Chauncey Marsh site for *S. regia*.

Table 3. Germination percentages for *Silene regia* seeds collected from four sites on two harvest dates when stratified for 12 weeks.

	Stratification began 10/19/99		Control	
Site	Aug 9	Aug 19	Aug 9	Aug 19
Allison Prairie	100 ± 0 a [1,2]	60 ± 35 a	33 ± 23 a	13 ± 23 a
County Road	93 ± 12 a	53 ± 12 a	47 ± 31 a	33 ± 31 a
Cemetery	53 ± 31 b	87 ± 23 a	27 ± 31 a	47 ± 12 a
Chauncey Marsh	27 ± 23 b	47 ± 31 a	7 ± 12 a	40 ± 35 a

	Stratification began 11/19/99		Control	
Site	Aug 9	Aug 19	Aug 9	Aug 19
Allison Prairie	87 ± 12 a [3]	73 ± 23 a [4]	67 ± 12 a	20 ± 20 a
County Road	73 ± 23 a	40 ± 20 b	73 ± 23 a	47 ± 12 a
Cemetery	47 ± 12 a	73 ± 12 a	33 ± 12 a	53 ± 23 a
Chauncey Marsh	53 ± 31 a	47 ± 12 ab	60 ± 35 a	40 ± 20 a

[1] Mean ± standard deviation. Means for October initiation followed by different letters within a column are significantly different (Duncan's multiple range test, 5% level).

[2] Means for October initiation of stratified seeds were significantly higher than control seeds within a collection date and stratification date based upon two-way analysis of variance at 5% level.

[3] For seed collected on August 9, means for November initiation of stratified seed were significantly higher than control seeds at all sites, excluding County Road, based upon one-way analysis of variance at 5% level, which was conducted due to a significant interaction between site and stratification treatment.

[4] For seed collected on August 19, means for November initiation of stratified seed were not significantly different than for control seeds based upon two-way analysis of variance at 5% level.

Table 4. Germination percentages for *Silene regia* seeds collected from four sites on two harvest dates when stratified for 15 weeks.

	Stratification began 10/19/99		Control	
Site	Aug 9	Aug 19	Aug 9	Aug 19
Allison Prairie	100 ± 0 a [1,2]	60 ± 20 a [3]	53 ± 42 a	7 ± 12 a
County Road	67 ± 23 b	60 ± 0 a	67 ± 12 a	40 ± 20 a
Cemetery	73 ± 12 b	73 ± 12 a	13 ± 23 a	40 ± 20 a
Chauncey Marsh	7 ± 12 c	33 ± 23 a	13 ± 12 a	13 ± 12 a

	Stratification began 11/19/99		Control	
Site	Aug 9	Aug 19	Aug 9	Aug 19
Allison Prairie	93 ± 12 a [4]	53 ± 23 a [5]	47 ± 12 b	33 ± 12 a
County Road	60 ± 0 b	47 ± 12 a	87 ± 23 a	33 ± 12 a
Cemetery	60 ± 0 b	33 ± 12 ab	40 ± 0 b	67 ± 31 a
Chauncey Marsh	20 ± 20 c	20 ± 0 b	60 ± 0 b	27 ± 12 a

[1] Mean ± standard deviation. Means for October initiation followed by different letters within a column are significantly different (Duncan's multiple range test, 5% level).

[2] For seed collected on August 9, means for October initiation of stratified seed were significantly higher than control seeds only at the Cemetery site, based upon one-way analysis of variance at 5% level, which was conducted due to a significant interaction between site and stratification treatment.

[3] For seed collected on August 19, means for October initiation of stratified seed were significantly higher than for control seeds based upon two-way analysis of variance at 5% level.

[4] For seed collected on August 9, means for November initiation of stratified seed were not significantly different than control seeds based upon two-way analysis of variance at 5% level.

[5] For seed collected on August 19, means for November initiation of stratified seed were significantly higher than control seeds, based upon two-way analysis of variance at 5% level.

Table 2 reveals a significant site effect for scarified seeds of *S. regia*, in that Chauncey Marsh seeds had lower percent germination than seeds from the County Road site. A significant location effect also was revealed in the non-scarified seeds where germination of seeds for the Cemetery site was higher than all other sites.

Stratification

Tables 3 and 4 demonstrate that *Silene regia* seeds collected on August 9 and August 19 had variable final germination percentages following stratification for twelve weeks or fifteen weeks with different stratification start dates. Some sites yielded higher percent germination within a stratification treatment, yet few consistent patterns occurred across sites, except for frequently lower germination of seeds from Chauncey Marsh as well as higher germination of seeds from Allison Prairie within many stratification treatments. These site differences were more notable in stratified than control seeds. Differences between control and stratified seed germination within a site also were inconsistent; however, when they were different, stratified seed had a higher germination than control seed. Moreover, no apparent differences were observed in germination of *S. regia* seed related to stratification start date or duration.

DISCUSSION

Silene regia seeds showed dormancy that was partially broken by several factors including scarification, stratification and after-ripening, although 100% germination rarely was achieved. None of these factors were an absolute requirement for germination. Rather, each of these techniques enhanced germination to varying degrees. These findings do not agree with literature on seed germination of *S. regia* in nature (Baskin and Baskin 1988, Menges 1991, Menges 1995, Baskin and Baskin 1998) that suggest stratification is required to break dormancy. Previous reports do not address the influence of scarification on these seeds. In the present study, germination occurred for seeds that were scarified, but were not stratified. In addition, in the present study, control seeds germinated when after-ripened even without stratification, unlike previous studies (Baskin and Baskin 1988). When the seed initially was collected, twenty seeds were used in a trial germination study in early fall 1999. None of the seeds germinated within two weeks. Further investigation is needed to document this effect more completely, since the present study was not designed to test after-ripening. These results suggest that the growth potential of freshly matured seed is insufficient to germinate without additional maturation, or elimination of the mechanical restriction of the seed coat. Since these various techniques all enhanced germination, the dormancy of these seeds may be related to both mechanical (seed coat) and physiological (embryo) factors.

Dormancy in seed of *S. regia* was more complex than for seed of *S. officinalis* as dormancy of *S. officinalis* was broken completely by scarification, suggesting dormancy is controlled primarily by the seed coat. These results are consistent with the preliminary germination tests of Lubke and Cavers (1969) who found that scarification of *S. officinalis* by nicking the seed coat yielded 100% germination. For *S. regia*, both seed coat and embryo factors likely were involved, whereas for *S. officinalis*, only seed coat factors likely were involved. However, other seed factors such as seed set, distribution, longevity, and herbivory (Menges 1995, Edgin et al. 2003) also may influence the success of these two species.

Factors other than dormancy also may be affecting the germination of *Silene regia* seeds. One factor may be the location where the plants were grown. Although considerable variation occurred, germination usually was lowest for seed from Chauncey Marsh and highest for seed from Allison Prairie as compared to County Road and Cemetery. Both Chauncey Marsh and Allison Prairie had smaller population sizes in comparison to County Road and Cemetery, so seed germination was not related to population size. Seed from Chauncey Marsh had the lowest seed masses, suggesting a correlation with germination percentage and seed mass. Plants at Allison Prairie are in a gravel prairie restoration dominated by sparse clump-forming grasses. Cemetery and County Road plants are along roadsides. These three sites all receive strong direct sunlight throughout the day and have relatively little competition. Plants at Chauncey Marsh are in a dense stand of big blue stem (*Andropogon gerardii*) which may increase shading, reduce nutrient availability, and inhibit successful location of the plants by pollinators.

Another factor is the date that the seeds were collected. Germination of *Silene regia* seed showed no consistent pattern relative to these dates i.e., seed from one date did not always have higher germination than seed from another date. However, significant differences between dates were observed. Seed color also varied on different collection dates and sites. Seeds collected on different dates may represent different maturities, and seed maturity affects germination (Baskin and Baskin 1998).

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