

Seed Germination in the Riparian Zone: The Case of the Rare Shrub, *Spiraea virginiana* (Rosaceae)

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ABSTRACT Conservation of rare species often involves restoration efforts to enhance or expand existing populations, a critical step of which is seed germination. Seed germination can be especially problematic in riparian zones where habitat disturbance through flooding can be a regular and frequent event. *Spiraea virginiana* Britton, a riparian clonal shrub, is a rare species that is particularly susceptible to problems at the seed stage; it is generally assumed to lack effective seed recruitment, although seeds are sometimes observed in the field. Therefore, we examined the effects of growth substrate and cold stratification on the number of days required for seeds to germinate and overall seed germination success. Overall, total seed germination for *S. virginiana* was low at only 10% and cold stratification had a significant effect on the initiation of germination; as the number of days of cold stratification increased, the time necessary for seeds to germinate decreased. Although seedlings of *S. virginiana* have not been observed in nature, this study indicates that seeds can germinate and thus recruitment by seed is at least theoretically possible. We recommend that a seed germination protocol using sterile soil with cold (5°C), stratification treatment of at least 30 d should be used in future propagation and restoration efforts.

Key words: Clonal, conservation, rare, restoration, seed germination, *Spiraea virginiana*.

INTRODUCTION Conservation of rare plant species often involves a variety of approaches, including habitat restoration, protection from large herbivores, control of plant competitors, or the outplanting of individuals to supplement existing populations or to create entirely new populations in restored habitats (Falk 1992, Jusaitis 2005). This last approach may consist of sowing seeds directly into the soil to mimic natural recruitment; native soil in particular is often expected to result in optimal plant performance (e.g., Ingham et al. 1985, Laakso and Setälä 1999). But for some species, greater success is achieved if seeds are germinated under optimal greenhouse conditions before seedlings are transplanted into the field (Huenneke 1991, Jusaitis et al. 2004, Wendelberger et al. 2007). The latter technique might also be preferred in cases of rare species for

which seed is not plentiful or multiple seed dormancy mechanisms exist. For example, threatened orchid species are often collected and grown ex situ for translocation to their natural habitat (Ramsey and Dixon 2003). Therefore, a key factor in ensuring the success of conservation efforts is knowledge of the specific germination requirements of the target species, including growth media, which can have a large effect in germination success (Lesica 1992, Jusaitis et al. 2004).

Seed germination has been well studied for decades (e.g., Koller et al. 1962, Thompson et al. 1977, Egley 1986, Bewley 1997, Baskin and Baskin 1998) and consequently there is a wealth of information available for many plants, yet relatively little is known about species that inhabit certain extreme environments. One such case is the riparian zone along streams and rivers, in which plant species are subjected to frequent disturbance resulting from sudden flooding following substantial precipitation events (Naiman and Décamps 1997). In some cases, an influx of a large amount of water can

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cause severe scouring of banks along meandering streams, resulting in the complete removal of all vegetation and exposure of the bare soil. Many plant species in the riparian zone have become adapted for these disturbances by having wind- and water-adapted propagules, resprouting capabilities, or the ability to withstand flooding (Naiman and Décamps 1997). However, this habitat can also be extremely difficult for seed germination and establishment during periods of flooding (Gregory et al. 1991). Restoration efforts aimed at riparian species must involve careful consideration of the seed germination requirements to ensure continued success.

To facilitate ongoing restoration efforts of a plant species in the riparian zone and provide information that may be helpful for other riparian species, we examined the seed germination requirements of *Spiraea virginiana* Britton, a rare shrub in the Rosaceae, which is restricted to stream banks. The species is listed by the United States government as threatened and is considered in peril in any state in which it resides due to its habitat specificity (USFWS 1990). Previous research has shown that this species is prolifically clonal (Brzyski and Culley 2011) and no seedlings have ever been identified in the natural habitat (Ogle 1991). There are presently no data evaluating seed recruitment potential for this species, as collection of seeds was previously restricted due to its threatened status. Seeds can occur (Brzyski and Culley 2011), although there are few seeds produced on plants, and they mature in the late summer to early fall. In temperate areas, species that mature fruits in autumn are generally expected to require some level of cold stratification for seed germination to mimic winter conditions (Baskin and Baskin 1998).

For this study, we used a factorial design to quantify the optimal growth media and temperature conditions promoting seed germination. We tested a few of the most common growth media used in germination experiments, including native field soil, which will provide evidence of germination potential in nature. We also tested the effect of moisture availability for a subset of treatments. For each combination of treatments, we measured days to germination (based on days after planting) and percent germination over a 2-wk period to determine recruitment potential for *S. virginiana*. In

addition to providing data on overall germination success of a riparian species, a protocol was developed that provides the optimum germination conditions for *S. virginiana*. This research will facilitate future propagation efforts and is directly applicable to the recovery of this threatened species in the wild.

METHODS

Study Species

Spiraea virginiana is a perennial rhizomatous shrub endemic to Southern Appalachia in the United States, where it occurs in the following seven states: Georgia, Kentucky, North Carolina, Ohio, Tennessee, Virginia, and West Virginia (USFWS 1992). The species grows 0.6–3 m tall with arching, upright stems, and the leaf shape is mostly oval with a serrate margin (USFWS 1992). Flowers are grouped in compound corymbs, which are white in color and appear between late May and late July. Flowers are visited by generalist pollinators, which are consistently observed on flowers, suggesting that pollinator limitation may not be present (Brzyski, unpubl. data). Seeds are small (2–3 mm in length), contain no endosperm, and are dispersed by gravity and water (USFWS 1992). Seeds begin to dehisce from their follicle between late August and September, presumably falling to the ground where it has been suggested that they do not form a long-lived seed bank (USFWS 1992).

Germination Experiment

Because pollen viability can play an important role in seed production within populations, pollen viability was first tested using pollen grains collected from five flowers from each of nine plants of *S. virginiana* in a natural population. Pollen grains were stained with Alexander's stain (Alexander 1980) to differentially stain the protoplasm and cellulose in the cell walls. The protoplasm is stained in viable pollen grains, whereas aborted pollen grains do not produce protoplasm and, as a result, do not stain. For each flower sampled, 200 pollen grains were examined under 10× magnification on a Nikon SMZ800 (Melville, NY) and categorized as viable or inviable.

Mature fruiting corymbs from naturally pollinated inflorescences of *S. virginiana* (voucher barcode 00227408, A) were collected from 13 plants located throughout a robust population in Scioto County, Ohio, between August and September 2008. Only after natural seed dispers-

al began, entire corymbs were carefully removed from the plant and each placed into a plastic bag. Capsules were removed immediately from the infructescence and stored in a cool, dry place at 15°C for 4 mo. Seeds were tested to determine if they imbibe water or if they are impermeable to water, following Baskin and Baskin (1998). In this procedure, a random sample of 10 seeds was placed in a petri dish on filter paper soaked with distilled water and monitored for 24–48 hr for water absorption based on visual appearance. This occurred in all seeds, thus indicating that scarification was not needed (Bansal et al. 1980). To ensure that the storage had no significant effect on future germination, a subset of seeds were germinated immediately after collection, before storage. In addition, a subset of seeds were tested for viability after storage using 1% 2,3,5 triphenyl tetrazolium chloride solution (Cottrell 1947). Hydrogen ions are released from viable embryos during respiration, which combines with the tetrazolium to produce a pink color in the embryo.

For the germination tests, seeds were first removed from their enclosed capsules (if necessary) and then randomly placed into the factorial design in which one of four growth substrates were combined with one of five cold treatments, either with or without moisture. There were four cold stratification treatments at 5°C for differing numbers of days (0 d, 30 d, 60 d, 90 d), one cold, dry temperature treatment at 5°C (90 d), and the control (15°C), which was dry and received no cold stratification. Seeds were also sown in four different growth substrates: field soil, autoclaved field soil (120°C for 20 min), sterile vermiculite, and filter paper. Field soil was collected from sites where *S. virginiana* naturally occurs. A separate control consisted of field soil without any seeds sown in it to test whether any germination occurred from a seed bank. Five replicates of 10 seeds were sown into each factorial treatment and all treatments were performed for a total of 1,000 seeds. Planted seeds were randomly positioned in a plastic bag mesocosm to prevent desiccation and placed under a timed grow light with a 12-hr light/dark cycle. After planting and positioning in mesocosms, moisture levels (well-watered) and temperature (approximately 22°C) were held constant during the germination phase of 2 wk.

Due to low germination success, the data did not meet statistical assumptions of normality and variance. Consequently, a nonparametric Kruskal-Wallis test was used to test the main effects of stratification and growth substrate on days to germination and percent germination using JMP 8.0 (SAS Institute, Cary, North Carolina). To analyze the interactions between the variables of cold stratification treatment and growth substrate, a *G*-test of independence was calculated with the Williams correction, which is recommended for a more conservative result (Sokal and Rohlf 1995).

RESULTS Tests of pollen viability revealed that pollen grains of *S. virginiana* collected from the field exhibited high viability of approximately 90%. Out of the 1,000 seeds sown across all treatments, only 103 (10.3%) seeds germinated. No *S. virginiana* seeds germinated from the control field soil, suggesting that any germination of *S. virginiana* seeds in the treatments were not due to a seed bank. In addition, the values reported here were consistent with the separate subset of seeds that were germinated immediately after collection (10% based on 10 seeds), and tested for viability after storage (100% based on 100 seeds), indicating that subsequent storage did not have a significant effect on either viability or germination.

Seeds germinated within all treatments, with the greatest proportion doing so early in the experiment; 40% of the germinated *S. virginiana* seeds emerged on their first day of germination, which was 4–11 d after planting, depending on the treatment (Table 1). Growth media had no effect on days to germination, but there was an effect of cold stratification (Kruskal-Wallis; $H = 39.82$, $df = 4$, $p < 0.001$; Table 1). The initial start of germination decreased as cold stratification interval increased with seeds within the moist treatments germinated on average 6.4 d earlier than those in the dry treatments.

In terms of total percentage of successful seed germination, there was a significant interaction between growth substrate and cold stratification ($G_{adj} = 26.31$, $df = 12$, $p < 0.01$) although the main effects were not individually significant (Figure 1). There was no consistent pattern and the top germination treatments were: 30-d stratification treatment on vermiculite (30%), dry and cold temperature on the vermiculite (22%) and autoclaved soil (20%), and 90-d stratification on field soil (16%).

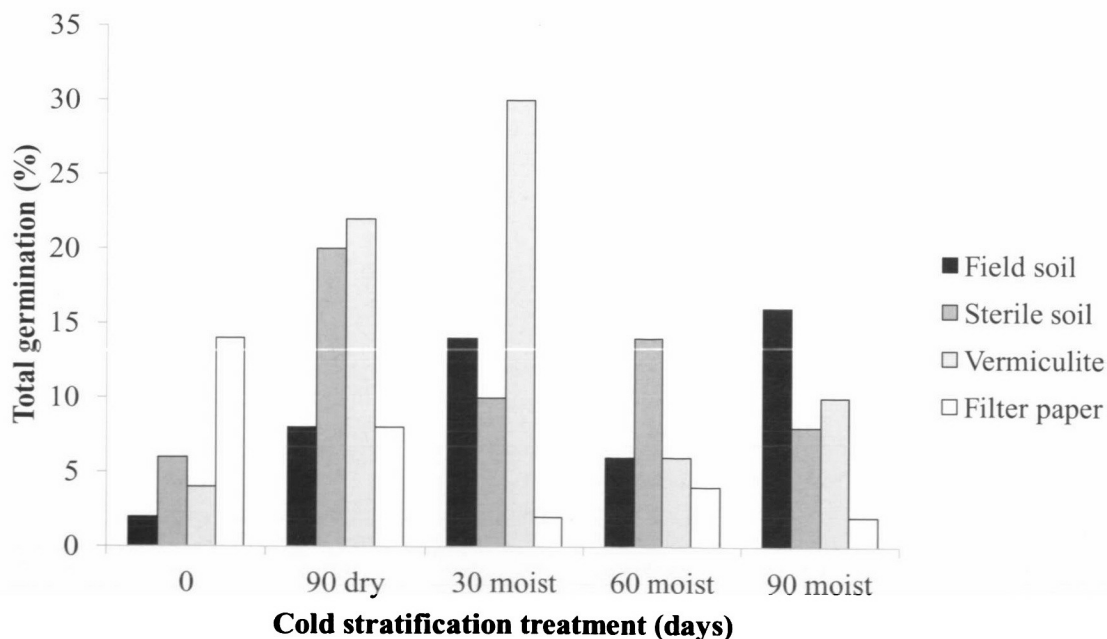
Table 1. Percentage of *Spiraea virginiana* seeds that germinated and when germination occurred (number of days after planting) following cold treatments (dry or moist)

Day until first germination	Treatment Conditions (days of cold—dry or moist)				
	0	90-dry	30-moist	60-moist	90-moist
4	—	—	—	2.9	6.8
5	—	—	—	5.8	—
6	—	—	1.0	—	—
7	—	—	9.7	3.9	7.8
8	—	—	9.7	—	—
10	—	—	—	1.9	—
11	7.8	21.4	4.9	—	1.9
13	1.9	2.9	1.9	—	—
15	1.9	1.9	—	—	—
18	—	2.9	—	—	—
20	1.0	—	—	—	—
Total	12.6	29.1	27.2	14.6	16.5

DISCUSSION Riparian species often grow in a challenging and frequently disturbed habitat, in which seed germination may be a critical factor for the persistence of natural populations. This is especially true for the restoration and conservation of populations of rare species, such as *S. virginiana*. Therefore, identifying the critical factors influencing seed

germination and seedling establishment is essential for long-term population persistence. In our study, we examined pollen viability and the effects of the growth substrate as well as stratification requirements on overall seed germination and days to germination.

Pollen viability testing demonstrated that *S. virginiana* pollen has high germination capacity

**Figure 1.** Total percent germination of seeds in different growth substrate for each cold treatment (dry or moist) for *Spiraea virginiana*. Growth substrates consisted of field soil (black bars), sterilized field soil (gray bars), vermiculite (light gray bars), and filter paper (white bars). Error bars are not shown as the data represent total seed germination per growth substrate.

and is not a factor in limiting germination. After testing the pollen in several different plants, we found that viability was consistently high (90%).

As predicted from the observation that species producing fruit in autumn should require some level of cold stratification (Baskin and Baskin 1998), *S. virginiana*, whose fruits mature between late August and early September, experienced faster germination with cold stratification. Days to germination shortened as the length of cold stratification increased, with the seeds experiencing the most days of cold stratification germinating >6 d earlier than other treatments. As expected for a riparian species, seeds subjected to cold temperatures and no moisture stratification exhibited no difference in days to germination when compared to the control, indicating that some level of moisture is important to germination time and may provide a signal to initiate germination. Such a signal would be beneficial in the natural environment, particularly in flood-disturbed habitat where timing is essential for seed germination (Blom 1999, Cooper et al. 2003). For example, rapid germination would be beneficial after a flooding event as this would allow quick establishment without competition from other plant species in the flood-scoured areas (Eriksson 1989, Cooper et al. 1999, Gerard et al. 2008). Any disturbance in the riparian zone would increase the amount of sunlight (Richardson et al. 2007), creating a more hospitable environment for seeds of riparian species such as *S. virginiana* to germinate. It is also interesting to note that germination virtually ceased in the stratification treatments after 11 d. Although moisture decreases the time to germination, it is possible that prolonged moisture may also hinder later germination due to possible deterioration of the seeds, and this is a factor that should be addressed in the future.

Growth substrate had no significant effect on seed germination. Soil quality can directly impact germination and establishment potential of plant species (Lesica 1992, Jusaitis et al. 2004), with the expectation that many plants will perform best in their native soil (e.g., Ingham et al. 1985, Laakso and Setälä 1999), although there are exceptions where higher germination occurs on other substrates (e.g., van Baalen 1982, Walmsley and Davy 1997). There was however, a significant interaction effect of growth substrate and cold stratification but with no

consistent pattern. Native soil did not result in the highest germination but when examining the environment most likely encountered in nature by *S. virginiana*, the 90-d stratification treatment, a majority of those seeds germinated in the natural field soil. Future research should expand upon this result, especially by increasing sample sizes to determine if growth substrate is a limiting factor for seed germination in *S. virginiana*. Additionally investigators should also explore the role of water availability so as to mimic natural flood-stage conditions in the riparian zone.

The rather low seed germination rate for *S. virginiana* quantified in this study (10%) is consistent with anecdotal accounts indicating a lack of *S. virginiana* seedlings detected in the field (Ogle 1991). Some rare species have good seed production and germination rates (Baskin et al. 1993, Aparicio and Guisande 1997, Lorite et al. 2007, Kadis and Georgiou 2010), but our research is in line with those studies where rare species experience a low level of recruitment (Simon and Hay 2003, Mendez et al. 2004, Young et al. 2007, García 2008, Copete et al. 2009, Skogen et al. 2010, Weekley et al. 2010). Our data are also consistent with low seedling recruitment often documented in clonal species (Eriksson 1989, Callaghan et al. 1992, Clark-Tapia and Molina-Freaner 2004). Despite the low seed germination, these results confirm that *S. virginiana* is not sexually sterile, and seeds can develop and germinate following natural pollination of inflorescences in the field. Low seed germination and subsequent low recruitment is also consistent with the discovery that populations are not genetically diverse; in fact, only three multilocus genotypes were identified at this specific population (Brzyski and Culley 2011). Although low genetic diversity is representative of other populations throughout the distribution of *S. virginiana* (Brzyski and Culley 2011), using only one population to test germination is a limitation to this study. Nonetheless, it is hopeful for this species that successful pollination and viable seed production did occur within a natural setting. Any amount of recruitment by sexually reproduced seed, however small, can prevent the complete erosion of genetic variation over generations (Soane and Watkinson 1979, Watkinson and Powell 1993). However, these positive effects are interpreted cautiously since seed germination success is

only one step relating to survival to reproductive maturity.

We suspect that seed recruitment, although probably rare, is occurring in *S. virginiana* and has just not been empirically observed. It is also likely in this clonal species that any seedlings will be directly competing with clonal shoots, which have an advantage because clonal shoots grow faster than seedlings (Bond and Midgley 2001). Future research should examine the growth rate and competition abilities between clonal ramets and sexually produced seeds in *S. virginiana*, as well as competition with other plant species capable of quickly colonizing the riparian zone. Overall, this study has shown that germination of naturally pollinated *S. virginiana* seeds is possible. To promote further propagation of the rare *S. virginiana* within a controlled environment, we recommend using sterile substrate and applying a cold (5°C) stratification treatment of at least 30 d. Restoration efforts would benefit best by germinating seeds first in a greenhouse and then transplanting them into the natural environment to minimize detrimental effects from biotic factors, such as competition, or abiotic factors associated with flooding.

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