



Ecology of *Salix variegata* seed germination: Implications for species distribution and conservation in the Three Gorges region



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ABSTRACT

The Three Gorges Project in China has brought significant changes to the region's riparian environment, posing serious challenges to biodiversity conservation. *Salix variegata* is a native shrub distributed on riverbanks in the Three Gorges reservoir region, and has important ecological function in the community. In this study, we studied the ecology of seed germination of *S. variegata* as efforts to understand its spatial distribution and develop conservation strategies. We found that soil type, soil moisture content and temperature all had significant effects on seed germination in *S. variegata*. Germination rate increased with the increasing soil moisture, while decreased in over-saturated soil. The germination rate in riverbank sand was higher than in sandy soil and clay loam. The optimal temperature for seed germination was 24 °C, under which 100% of germination was reached in 7 days. Seed viability and germination declined significantly with time after being released from fruits. The results suggest that soil moisture is the determining factor on seed germination, and influenced distribution of *S. variegata*. The tributary riverbanks above 175 m in the Three Gorges reservoir region can be used for the conservation of the species, as their fluvial patterns should have not been disturbed by the hydropower project.

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1. Introduction

Seed germination and seedling establishment are critical stages in the plant's life cycle, and to a large extent, they determine the dynamics and distribution of plant populations. Seed germination and seedling establishment are also the most vulnerable stages to environmental disturbances, and associated with extremely high mortality rates (Moles and Westby, 2004; Abe et al., 2008). River regulation and water reclamation projects have altered the fluvial patterns of rivers worldwide (Nilsson et al., 2005; Leyer, 2005). The constructions of dams and diversions usually result in modifications of the timing, magnitude and frequency of peak flows, reduce sediment loads, and cause abrupt water table declines. These alterations negatively impact the life cycle of flow-dependent riparian plant species, thereby resulting in recruitment failure and overall decline of a riparian vegetation (Merritt and Cooper, 2000; Braatne et al., 2007). Characterizing the ecological impact of these disturbances on seed germination will not only lead to the understanding in effects of water reclamation projects on the distribution patterns of riparian plants, but also to the development of conservation strategies to mitigate the negative impact of the disturbance (Manfred et al., 2004).

Salix variegata (Salicaceae), a peculiar perennial shrub to China, is restricted to the riverbanks of the Yangtze River in the Three Gorges region and other rivers in Southwest China (Chen et al., 2008). *S. variegata* grows new foliage usually in September after being submerged for

3–6 months, flowers and sets fruits from October to April in the following year depending on plant emergence time. It usually produces a large amount of small seeds (1–1.5 mm in length) with pappus which facilitates seed dispersal via wind and/or water flow. Seed germination occurs shortly after seeds are released into a favorable environment such as a sandy beach. Seedlings only exist on riverbanks and therefore the species is restricted to riverbanks, though seeds are capable of being dispersed over long distance by wind or flooding.

The Three Gorges Project, one of the biggest hydropower projects in the world, has brought significant changes to the region's riparian environment characterized by wide fluctuation and a reverse in the seasonal fluvial pattern. The water level of the Three Gorges reservoir now rises to 175 m in winter and retreats to 145 m in summer. The seasonal hydrological environment of riverbanks also has been changed from summer flooding with winter drought to summer drought with winter flooding in the reservoir (Chen et al., 2005). The environmental changes pose serious challenges to biodiversity conservation in the region. The rising water level inevitably leads to the submersion of vast area of riparian habitats. Meanwhile, the drought and high temperature in summer and flooding in winter have been detrimental to seed germination in many plants, and also to seedling establishment, growth and development.

In this study, we examined the effects of environmental factors on seed germination in *S. variegata* by addressing the following specific questions: (1) Why is *S. variegata* regeneration limited on the riverbanks? (2) Apart from submersion due to the Three Gorges Project are there any environmental factors negatively impacting the establishment of *S. variegata* in the reservoir area? Implications for conservation were discussed in light of the research results.

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2. Materials and methods

2.1. Experimental materials

Hundreds of *S. variegata* plants were transplanted to the Ecology Experimental Garden at the Three Gorges University from the Three Gorges reservoir region before their habitats were submerged in 2003. Inflorescences were collected from the transplanted plants and used for germination experiments in October 2011.

2.2. Experimental design

Environmental factors that may directly influence seed germination of riparian plants include soil moisture, soil type and temperature (Chen and Xie, 2007). Controlled experiments were implemented to test the effects of these three soil parameters on seed germination. Three types of soil, seven soil moisture regimes and eight temperature treatments, each with five replicates, were tested. Soil was collected from the reservoir area. The three soil types were bank sand, sandy loam and clayey loam collected respectively from the river bottom, riverbank and abandoned farmland on the upland where the seeds of *S. variegata* may disperse. The experimental soil water content was set from 5.0 to 41.0% at 6.0% intervals. Germination temperature was tested from 12 to 33 °C with 3 °C increments. The experimental unit consisted of 50 mature seeds placed on a Petri dish filled with 50 g of soil substrate, and was tested with different soil, temperature and soil moisture combinations. The Petri dishes were covered with perforated caps. Due to the small size of the seeds, gauze was attached to the cap to prevent seed loss.

One hundred mature seeds were placed on a sandy loam substrate with saturated soil water content and germinated at 24 °C (the optimal combinations of germination condition in the above experiment) to investigate the germination dynamics and viability change of seeds over time. Five replicates were employed. Hundreds of inflorescences were collected and stored under room conditions (temperature 13–23 °C, humidity 75%). One hundred mature seeds were daily released artificially from fruits and germinated under the optimal combinations of germination condition to observe the change in seed viability over time. Five replicates were employed.

All germination trials were performed in a growth chamber under 12:12 h light to dark cycle. Soil water content was measured and maintained daily. Germinated seeds were counted and removed on a daily basis. The experiment was terminated when no germinated seeds were observed after two successive counts.

2.3. Data analysis

Petri dishes were considered as the unit of replication and measurements for individual plants that were averaged within the Petri dishes. Percentage of germination was calculated for all tests. A univariate general linear model analysis was conducted with the dependent variable '% seed germination' and fixed factors 'soil type', 'soil water content', and 'temperature' to investigate the effects of soil type, soil water content, and temperature on seed germination. One-way analysis of variance (ANOVA) was performed to determine the dynamics of seed germination and the changes of seed viability over time. All analyses were conducted using an SPSS software (13.0).

3. Results

3.1. The effects of ecological factors on seed germination

Univariate tests showed that the individual treatments of soil type, soil water content, temperature, and their interaction had significant effects on seed germination (Table 1).

Seed germination rate increased with temperature increasing from 12 to 24 °C, and declined at temperature higher than this. The highest

Table 1

Univariate analysis of the effects of three ecological factors on germination of *Salix variegata* seeds.

Source	df	Mean square	F	Sig.
Temperature	7	12926.637	420.053	0.000
Soil water content	6	14724.105	478.462	0.000
Soil type	2	4586.671	149.045	0.000
Temperature * Soil water content	42	243.141	7.901	0.000
Temperature * Soil type	14	378.296	12.293	0.000
Soil type * Soil water content	12	543.888	17.674	0.000
Temperature * Soil type * Soil water content	84	118.503	3.851	0.000

average germination rate (60.6%) was recorded at 24 °C. An LSD test showed that the optimum temperature range for seed germination was 21–24 °C (Fig. 1).

Germination rate of *S. variegata* seeds differed significantly among soil types ($P < 0.01$). The average germination rates 45.8% and 45.3% in sand and sandy loam substrate, respectively, and both were significantly higher than that in clayey loam substrate (Fig. 2).

S. variegata seeds germinated at a wide range of soil moisture contents (5 to 41%). Germination rate increased with increasing soil moisture content, reaching the highest value in moisture saturated soil (Fig. 3). An LSD test showed that the optimum soil water content for seed germination was the saturated soil moisture content, which differed among soil types (Fig. 4).

3.2. Seed germination dynamics and seed viability over time

Over 70% of *S. variegata* seeds germinated within 3 days on saturated soils at 24 °C. All seeds germinated within seven days (Fig. 5). The viability and germination of *S. variegata* seeds that were stored under room conditions declined dramatically after being released from fruit ($P < 0.01$). Over 94.6% of seeds that were stored one day and subjected to test germinated, while only 5.3% of those seeds that were stored nine days germinated (Fig. 6).

4. Discussion

Riparian *Salix* species have similar life-history traits. They are dioecious, usually flower and set seed in spring; they produce a large number of small hairy seeds, and disperse them soon after the seed matures; seeds germinate rapidly after being released, or perish shortly thereafter (Niiyama, 2008). A single mature plant of *S. variegata* is capable of producing thousands of non-dormant tiny seeds annually, while seeds are only viable for 9 days and germinate mostly within 3 days if they dispersed to a suitable environment. Riparian plant species may experience environmental stresses during seed dispersion and germination. As a result, it is likely that only a small proportion of seeds could reach a suitable microsite for germination and establishment during the limited 'time window' (Mahoney and Rood, 1998). High seed production may have a strong fitness advantage in occupying habitat gaps in highly disturbed and unpredictable environments (Crawley, 1997).

The results revealed that temperature, soil water content and soil type have significant influences on the germination of *S. variegata* seeds. *S. variegata* seeds mature in autumn. Atmosphere temperature during that time in the Three Gorges reservoir region is usually higher than 20 °C, so temperature is not likely the dominant factor limiting seed germination and species distribution of *S. variegata*. The results also imply that soil type also is not likely to limit the germination of *S. variegata*, as there was no significant difference in germination between the substrates under a moisture saturated condition. Consequently, it can be concluded that soil moisture content and the interactive effects of soil moisture content and soil type would play crucial roles in determining seed germination and species distribution of *S. variegata*. Few *S. variegata* seeds germinated when soil water content was below 5%,

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