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Coping with stressful environments: An experimental study of seed germination and seedling survival of Mexican riverweeds under natural conditions

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A B S T R A C T

Seed germination and seedling survival are fundamental processes in the life cycle of Podostemaceae, the largest family of strictly aquatic angiosperms. However, few studies to date have addressed seed germination and seedling survival of Podostemaceae under natural conditions. To fill this gap, we performed a field experiment to study the seed germination and seedling survival of *Marathrum foeniculaceum* and *Noveloa coulteriana*, two species of Podostemaceae, in two rivers with contrasting light incidence, the Boca de Tomatlan (BT) and Las Juntas del Tuito (LJ) Rivers, in Jalisco, Mexico. We found significant differences in the final proportion of seeds germinated between *M. foeniculaceum* (67.74%) and *N. coulteriana* (32.5%), and the proportion of seeds germinated in the BT river (higher light incidence) was significantly higher than in the LJ river. Likewise, seeds germinated more quickly in *M. foeniculaceum* than in *N. coulteriana*, and in the BT river than in the LJ river. Final seedling survival of both *M. foeniculaceum and N. coulteriana* was rather low (2.53% and 1.08%, respectively), but no significant differences were detected between species or rivers. This paper represents one of the first studies to experimentally evaluate aspects of the ecophysiology of seed germination and seedling survival of Podostemaceae under natural conditions, and it contributes to our understanding of the ecology and conservation of this particular family of plants.

1. Introduction

Seed germination and seedling survival are fundamental processes determining population dynamics, genetic structure within and among plant populations, and species' ability to colonize new environments (Baskin and Baskin, 2014; Jersáková and Malinová, 2007). Seed germination is regulated by intrinsic factors, such as seed dormancy, and external factors, such as light quality and temperature (Baskin and Baskin, 2014), while seedling recruitment mainly depends on the probability that seeds will arrive to a suitable microhabitat (Nathan and Muller-Landau, 2000).

The Podostemaceae family is a particular group of strictly aquatic angiosperms (Philbrick and Novelo, 1995; Quiroz et al., 1997; Philbrick, 2004). Although recent evidence suggests that clonal reproduction is more frequent than previously thought (Philbrick et al., 2015), sexual reproduction is considered the main mode of reproduction in Podostemacae (Philbrick and Novelo, 1994; Philbrick and Novelo, 2004). The Podostemaceae species' life cycle is closely associated with the seasonality of the rivers they inhabit. During the rainy season, the vegetative structures of plants grow firmly attached to the rocky substrate of the rivers. Later on, during the dry season, the water level of the rivers drops, and flowering and fruiting occur. During this period, pollination occurs and seeds are produced (Luna et al., 2012). Seeds are then dispersed over the rocky substrate and will germinate the next rainy season, reestablishing the plant populations (Kato, 2013). Thus, seed germination and seedling survival are major processes in the life cycle of Podostemaceae, as they drive both the distribution and abundance of these plants (Philbrick and Novelo, 1995; Reyes-Ortega et al., 2009).

Despite their importance, few studies have addressed the analysis of seed germination and seedling survival in Podostemaceae. Most germination studies have been conducted in *in vitro* conditions to obtain seedlings to perform anatomical and embryological studies (Vidyashankari and Ram, 1987; Sehgal et al., 1993; Philbrick and Novelo, 1994; Uniyal and Ram, 1996; Ram and Sehgal, 1997).

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Seed germination experiments under laboratory conditions have shown that seeds of Podostemaceae are non-dormant and germinate abundantly (>80%) when supplied with light and water (Devi, 2014). Likewise, an *in vitro* study by Vidyashankari and Ram (1987) in the Asian Podostemaceae *Griffithella hookeriana* found a seedling survival of 64% 30 days after sowing and that seedling survival decreased as the storage period increased. The study also found low seedling survival (~0%) when seeds were stored at room temperature for a period of 12 months. Grubert (1976), studied germination of five Venezuelan Podostemaceae species under natural conditions, finding that germination is only achieved when seeds were exposed to light, and that seedling fixation to rocks under turbulent water is from weak to moderately high (25%–69%). Yet, information regarding seed germination and/or seedling survival rates under natural conditions in Podostemaceae is still scarce.

Empirical evidence on the ecological features of Podostemaceae is not only a matter of scientific interest but is also crucial for their conservation, which is an imminent problem worldwide (Kato, 2013). About one third (15–37%) of Neotropical Podostemad species are endemic to a particular river, and about 33% are two-river endemic species (Philbrick et al., 2010). Given this high degree of local species endemism, Podostemaceae biodiversity is extremely sensitive to habitat loss and disturbance and is threatened by the construction of hydroelectric dams and increased river pollution from agricultural and domestic activities (Cook and Rutishauser, 2007; Kelly et al., 2010; Ghogue, 2011). Seven species of Podostemaceae are found in Mexico; three of them are endemic to Mexican territory (*Marathrum plumosa, Noveloa longifolia*, and *Noveloa coulteriana*), most inhabiting clean, oligotrophic rivers (Quiroz et al., 1997; Novelo et al., 2009; Tippery et al., 2011). Philbrick and Novelo (1994) studied seed germination of four Mexican Podostemads species: *Marathrum foeniculaceum* (including *Marathrum haenkeanum* and *Marathrum rubrum*), *Noveloa coulteriana* (syn. Oserya coulteriana*), Marathrum plumosum* (syn. *Vanroyenella plumosa*), and *Tristicha trifaria* (see Tippery et al., 2011). All species showed high germination (>95%) after being stored for 18 months. Though, this work did not test germination requirements (temperature and light quality) for these species. Reyes-Ortega et al. (2009) and Castillo et al. (2013) studied in laboratory the light quality, temperature, and storage time requirements for germination of *Marathrum foeniculaceum* and *Noveloa coulteriana*. In both studies, the seeds were positive photoblastic (require light rich in red and poor in far red to germinate), thermoblastic (germination decreased at lower temperatures, and it is inhibited at 15° C), and showed high germination rates (~90%). In a recent study, Flores-Enríquez (2015), found no differences between the germination requirements and germination rates of *Marathrum foeniculaceum* and *Noveloa coulteriana* in laboratory conditions. Nowadays, information of seed germination and seedling survival of Mexican species of Podostemaceae under natural conditions is lacking. Generating such data is relevant not only to understand the Podostemad ecology, but also to design restoration strategies of this particular group of plants.

Here, we carried out a field experiment to study the seed germination and seedling survival of *M. foeniculaceum* and *N. coulteriana*, two New World species of Podostemaceae, in two different rivers in Mexico that contrast with one another in terms of light incidence due to canopy openness.

2. Methods

2.1. Plant species

Noveloa coulteriana (Tul.) C. Philbrick (syn. *Oserya coulteriana*) is a small herb, with filamentous leaves up to 10 cm long and 0.5–1.8 mm in diameter, annual or perennial, and with prostrated and flattened roots (Philbrick and Novelo, 1997). It is endemic to Mexico and is considered an endangered species by the Secretaria del Medio Ambiente y Recursos Naturales (2010). *N. coulteriana* is distributed along the Pacific Coast from Sonora to Guerrero and Baja California Sur (Philbrick and Novelo, 1997). *Marathrum foeniculaceum* Humb. & Bonpl. (also referred to in the literature as *Marathrum schiedeanum* or *Marathrum rubrum*, but see Tippery et al., 2011) is an annual or perennial herb, characterized by pinnately compound red to green leaves up to 20 cm long with prominent expanded central rachis, and repeatedly pinnate (Novelo and Philbrick, 1997). *M. foeniculaceum* is endemic to America, ranging from central Mexico to Colombia (Tippery et al., 2011). Both species are reported to inhabit clean, oligotrophic rivers. However, most of the rivers that these species inhabit in Mexico are exposed to some degree of anthropogenic disturbance (Quiroz et al., 1997; Reyes-Ortega et al., 2009).

2.2. Field experiment

During January–March 2016 (the end of the dry season in the region), we conducted a field experiment at two different rivers near Puerto Vallarta, Jalisco, in Mexico (Supplementary Fig. S1a). The first site was located in the Horcones River, near the town of Boca de Tomatlan, in the municipality of Puerto Vallarta (20°30′39. 1"N 105°18′51. 2"W; hereafter BT); the second was located in the Arroyo del Rincon River, in the Las Juntas del Tuito village in the Cabo Corrientes municipality (20°14′39. 6"N 105°18′51. 0"W; hereafter LJ). We performed a preliminary analysis of water chemistry (nitrites, nitrates and phosphates concentration) and pH during January 2016 in BT and LJ rivers using a Multiparameter Bench Photometer (C200, Hanna Instruments). Data on water chemistry rivers is described in the supplementary Table S2.

We included two experimental points in each river, spaced ~500 m apart. At each experimental point, we placed three replicates for each species. Replicates consisted of groups of seeds (200 seeds for *M. foeniculaceum* and 60 seeds for *N. coulteriana*) that were sowed independently into granite rocks (length: 20 × width: 20 × depth: 10 cm). In order to avoid artificially dispersing seeds between rivers, we used only seeds collected from the river where the site was located (i.e. seeds sowed at the BT river were from fruits collected from the BT river, and likewise for the LJ river). All fruits where collected in January 2015 and stored at room temperature until experiments were performed. To attach the seeds to the granite rocks, we took advantage of the sticky mucilage that is characteristic of the seed coat in Podostemaceae (Jäger-Zürn, 2000). Seeds were moistened with enough water to hydrate the mucilage, then arranged into a 10 × 10 cm grid and left to dry for one week before the field experiment; once the mucilage was dry, the seeds became strongly adhered to the rocks. This allowed us to place these rocks at the river field sites and carefully follow seed germination and seedling survival using a Leica MZ6 stereomicroscope. About 10% of the sowed seeds were lost between sowing and expected germination (5 days after sowing, based on laboratory germination experiments) in both sites and species. Statistical analyses were therefore performed on seeds that remained attached to the rocks five days after sowing (DAS). We recorded seed germination every two days for 17 DAS and calculated the daily seed germination percentage (DGP) and the final seed germination percentage (FGP). We also calculated the seedling survival percentage (SSP) at three different developmental stages: *(i)* germinated seeds (17 DAS), *(ii)* young seedlings (20 DAS), and *(iii)* late seedlings (60 DAS). In addition, we calculated the final seedling survival percentage (FSSP) at 60 DAS.