Methods

*Study Sites and Species*

*~Southwestern USA*

So noran Desert southwestern riparian ecosystems are located in the arid environment of Arizona USA. Rainfall follows a bimodal pattern reflecting the monsoon and Pacific frontal storm seasons, which produce approximately 29-36 cm of precipitation annually. Temperatures widely fluctuate in the Sonoran Desert with a mean low of 1°C and a mean high of 39°C.

A variety of plant communities exist along the declining moisture and flood-disturbance gradients that are typical of arid southwestern riparian ecosystems. *Prosopis* forests, *Hymenoclea* shrublands, *Populus-Salix* forests, and active channel bars develop along the hydrogradients (listed low to high) and are the dominant communities. In terms of size, with the exception of the active channel bars, woody species dominate the plant communities. However, most plant species that exist in these communities are annual and short-lived perennials species (Wolden et al. 1994, Bagstad et al. 2005). As is common in frequently disturbed communities, the herbaceous species produce longer-lived seeds capable of persisting in a seed bank (Howard and Lyon 1952, Boudell 2004). The dominant woody species in the *Populus-Salix* forests produce short-lived seeds that germinate rapidly after dispersal (Stromberg 1993, Shepperd 2008).

Most of the herbaceous and woody species we selected for study are common in southwestern riparian ecosystems. *Populus fremontii*, *Salix gooddingii*, and *Tamarix chinensis* are dominant woody species in the *Populus-Salix* wetland forests. The active channel bar and *Populus-Salix* wetland forests are frequently populated by the herbaceous species *Mimulus guttatus*, *Nasturtium officinale*, *Polypogon monspeliensis*, and *Veronica anagallis-aquatica* (Wolden et al. 1994). *Sisymbrium irio* is a common herbaceous species found in *Populus-Salix* and *Prosopis* forest communities (Wolden et al. 1994, Wolden and Stromberg 1997). All of our study species are native to the U.S.A., with the exceptions of *N. officinale*, *P. monspeliensis* *S. irio*, *T.* *chinensis* (Table 1).

Our common southwestern species also have varying life history traits (Table 2). The majority of the herbaceous plants in this study are cool season short-lived perennials. However, both *P. monspeliensis*, a cool season grass, and *S. irio*, a cool/warm season forb, are annuals. The woody species produce transient seeds that germinate shortly after dispersal in the spring (Stromberg 1993, Shepperd 2008). While the herbaceous species are seed-banking species that produce persistent to long-term persistent seeds (Howard and Lyon 1952, Boudell 2004).

*~Mid-East to Eastern USA*

Two of our study species, *Cephalanthus occidentalis* and *Alnus serrulata* are located in the mideastern to eastern USA (Table 1). Both species are native and are commonly found in wetland environments. *A. serrulata* is a riparian shrub-tree species found in riparian ecosystems of the Piedmont ecoregion. *C. occidentalis* is a shrub-tree species located in swamps. Like the other woody species in our study, they produce transient seeds (citation).

*Seed Collection*

Seeds of herbaceous and woody species were collected from various populations and individuals in the USA (Table 3). The long-lived seeds of our herbaceous species were collected in May 2010 from sites located along the San Pedro River, Hassayampa River, and Cienega Creek in Arizona, U.S.A. The short-lived woody species seeds were collected just prior to project initiation. Seeds of *A. serrulata* were collected in November 2012 from sites along Jesters Creek in Georgia, U.S.A. In April 2013, the seeds of *P. fremontii*, *S. gooddingii*, and *T. chinensis* were collected along the Salt River in Arizona, U.S.A. *C. occidentalis* seeds were collected in 2013 in . All seeds were stored in paper bags at 3°C until the start of the project. Before treatment application, seeds were removed from inflorescences, fruits, or cones (e.g., *A. serrulata*) and pappus removed (e.g., *P. fremontii*), if needed.

*Treatments*

*~Herbaceous Species Trials*

Herbaceous species trials began on June 11, 2012. Because seeds of the herbaceous species were very small, and we wanted to ensure that there were enough seedlings for the remainder of the study, varying amounts of seeds were used from at least 10 individuals per species in the germination trials (Table 3). For each herbaceous species, at least 25 seeds from at least 10 individuals and 1-3 populations were placed in the same orientation on pre-wetted 9 cm filter paper, which was sitting on top of sandy loam in small pots (Table 3, Figure X). Six pots per species were randomly assigned one of six water level x CO2 treatments (n = 6 per treatment, 36 total pots, at least 150 seeds per species) (Table 4). Pots were placed into bins and were subjected to the prescribed water treatments of high, low, or alternating water levels (Figure X). High water levels in the bins kept the soil saturated for high water treatment pots. In the same bin, low-level water treatments, which kept the soil moist-dry, were created by raising the low water treatment pots on concrete tiles. Alternating water levels were simulated in the same bin by raising the alternating water treatment pots on tiles every 3 days. The bins were then placed into growth chambers set at either ambient CO2 (370 ppm at time of study) or elevated CO2 (740 ppm) levels. Growth chambers were programmed with a diurnal light cycle of 12-hours of light. To encourage germination, all seeds were subjected to “spring” conditions, which provided a 14-hour photoperiod and 10 hours of high temperature at 22.8°C and 14 hours at 10°C for 8 weeks. Water levels were frequently monitored and adjusted to maintain treatment conditions. Pots were checked for germinants every 4 days.

The herbaceous species germination trial ended on June 26, 2012. Tetrazolium tests were conducted on ungerminated seeds. They were incubated for 24 h in ~5 mL of 1% 2,3,5-triphenyltetrazolium chloride solution. Pink embryos indicated a living seed and those embryos that did not stain or irregularly stained were considered dead.

*Woody Species Trials*

The woody species trial began on April 28 and 29, 2013. For each of the woody species, 25 seeds from at least 10 individuals and several populations were placed on wet 9 cm filter paper that was sitting on top of sandy loam soil in small pots (Table 3, Figure X). With the exception of *A. serrulata*, woody species followed the same treatment protocol as the herbaceous species. *A*. *serrulata* received only a low water treatment and either “ambient” or “elevated” CO2 levels. To encourage germination, all seeds were subjected to “spring” conditions, which provided a 14-hour photoperiod and 10 hours of high temperature at 22.8°C and 14 hours at 10°C for 8 weeks. Seeds that developed into seedlings were subjected to 13 weeks of “summer” conditions, which started at the end of “spring” and lasted until the end of the study. Summer conditions consisted of a 12-hour photoperiod and ramped temperatures ranging from a low of 18°C to a high of 30°C. To support plant growth, Hoagland’s nutrient solution, which contains macro and micronutrients, was added to all bins on June 11, 2013. During the germination trial, moldy filter paper was exchanged for new filter paper.

Due to the presence of rapid germinators in the study, pots were checked for germinants within 24 hours of project initiation and daily until the growth and establishment phase of the trial. A dissecting scope was used to assess germination on May 3 and 7, 2013. At the end of the germination phase of the trial, on May 15, 17, and 21, 2013, ungerminated seeds of *P. fremontii*, *S. gooddingii*, and *T. chinensis* were tested for viability using tetrazolium tests. Ungerminated seeds were incubated for 24 h in ~5 mL of 1% 2,3,5-triphenyltetrazolium chloride solution. Pink embryos indicated a living seed and those embryos that did not stain or irregularly stained were considered dead. INCLUDE VIABILITY DATA Germination data of *A*. *serrulata* and *C. occidentalis* were not included in the germination analysis, and so tetrazolium tests were not conducted, due to erratic and delayed germination. However, the seedlings that eventually germinated were included in the growth and establishment stage of the study.

Based on germination success and establishment, seedlings were removed from filter paper and planted in their pots. Due to rapid germination and growth, three seedlings of *P. fremontii*, *S. gooddingii*, and *T. chinensis* were transplanted to pots on May 20, 2013. These pots were thinned to one seedling on May 31 for *P. fremontii* and on July 16 for *S. gooddingii* and *T. chinensis*. *C. occidentalis* seedlings were thinned and transplanted to pots on July 16. *C. occidentalis* pots without seedlings received one seedling from other pots within the same treatment group. Seeds of *A. serrulata* did not germinate during the germination trial and were returned to cold storage (10°C) for an additional four weeks of cold stratification. On July 30, *A. serrulata* seeds were returned to their respective growth chambers where some seeds subsequently germinated.

Saplings were harvested and dried on October 21, 2013. Intact specimens were cold stored until January 14, 2014. On this date, the specimens were bisected into shoots and roots, which were subsequently weighed. The diameter and stem length were also measured. A few pots had several saplings, and in these cases, data were averaged for the pots.

Quantitative Assessments

Results

While germination trials typically last 30 days, germination was immediate for several woody species and so germinant counts began on the same day the trial began (April 30). *P. fremontii*, *S. gooddingii*, and *T. chinensis*

Due to lack of germination, *A. serrulata* seeds were subjected to an additional 4-week cold stratification treatment (June 26-July 30, 2012).

Every 5 days, germinants were counted, moldy seeds rinsed, moldy filter paper replaced, and treatments were reapplied.

At the end of the 30-day trial, ungerminated seeds were cold stored at 3°C and ten days later tetrazolium viability tests were conducted on the ungerminated seeds. Ungerminated seeds were treated with 4.5 ml of 1% 2,3,5-triphenyltetrazolium chloride in a pH 7 phosphate buffer solution and incubated at room temperature in the dark for 24 hours. Viable seeds contained pink-stained embryos, whereas dead seeds were unstained or unevenly stained.

Citations

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