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Capsule Treatments to Enhance Seedling Emergence of *Gaura neomexicana* ssp. *coloradensis*

Leah M. Burgess,^{1,2} Ann L. Hild,^{2,3} and Nancy L. Shaw⁴

Abstract

Management of riparian vegetation is difficult because these communities are frequently impacted by herbivores, invasive weeds, and altered hydrologic regimes. Multiple and intertwined factors affecting rare species recruitment are particularly difficult to identify. *Gaura neomexicana* ssp. *coloradensis* Munz (*Gaura*) is a short-lived perennial forb endemic to riparian areas in mixed-grass prairies of Wyoming, Nebraska, and Colorado, U.S.A. It became a federally listed threatened species in October 2000. Because the species is a recruitment-limited monocarpic perennial, we studied the effects of six capsule-collection dates, a 2-month cool-moist stratification, 24-hr leaching, and 24-hr imbibition on *Gaura* seedling emergence. Seedling emergence did not vary with collection date. Capsules collected from *Gaura* plants grown at the Bridger Plant

Materials Center in Montana exhibited greater emergence than capsules harvested from endemic populations near Cheyenne, Wyoming, suggesting that maternal plant growing conditions impact dormancy. Because cool-moist stratification enhanced seedling emergence of *Gaura* and leaching did not, sufficient moisture during cool temperatures may be more critical than leaching of germination inhibitors as might occur with normal stream flows. Spring flooding may enhance *Gaura* recruitment by increasing the availability of riparian sites that are inundated during periods of cool temperatures. If so, hydrologic and climatic regimes must be considered in restoring the unique conditions needed for germination of this rare riparian endemic.

Key words: endemic, germination, rare, riparian, stratification.

Introduction

The rare riparian endemic Colorado butterfly plant (*Gaura neomexicana* ssp. *coloradensis* Munz (Onagraceae)) (hereafter termed *Gaura*) became a federally listed threatened species under the U.S. Endangered Species Act in October 2000 (Jennings et al. 2000). To identify management practices favoring the emergence of this rare and little-documented forb, seedling emergence studies were conducted using seed from endemic *Gaura* populations at F. E. Warren Air Force Base (WAFB) near Cheyenne, Wyoming. *Gaura* is recruitment-limited and establishment to the rosette stage is considered critical in the life cycle of the species (Floyd & Ranker 1998). Hence, understanding factors regulating seedling emergence is of great importance in developing management strategies to favor *Gaura* recruitment.

Gaura is a short-lived, monocarpic perennial endemic to riparian corridors in northern mixed-grass prairies (Jennings et al. 2000). It remains in the rosette stage for the first 2–4 years before bolting, producing seed capsules,

and dying (Floyd & Ranker 1998). Seed capsules are fusiform, 2–3 mm thick, and four-angled with a median spine at each angle (Marriot 1987). Each capsule contains three to five seeds that are each enclosed within the flesh of the capsule (Burgess 2003). Capsules do not dehisce in dry storage.

Historically, flooding was an annual disturbance in *Gaura* habitats (Fertig 2000). Bare substrates with little shade or litter cover produced by flooding are favorable microsites for germination (Floyd 1995). As a result, flowering *Gaura* individuals typically increase in density 2 years after flooding (Heidel 2002). *Gaura*, a C3 species (Watson & Dallwitz 2002), grows and reproduces during the cooler months of the growing season. When this season is truncated during drought periods, as occurred from 1999 to 2003 at WAFB (Fig. 1), germination of *Gaura* may be reduced due to lack of moisture. *Gaura* seedling emergence and rosette establishment increase during cooler springs, and bolting *Gaura* increases 2 years after cool springs (Laursen & Heidel 2003). This suggests that cooler spring temperatures provide more favorable conditions for germination and rosette establishment.

Pregermination treatments were developed to discern the relationship of cool spring temperatures during imbibition and the potential leaching action of running water to *Gaura* seedling emergence. We hypothesized that stream flows (moisture as running water) may be required to leach germination inhibitors from capsules. Alternately, spring flooding and beaver ponding may provide extended

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Figure 1. Precipitation at WAFB (bars) from January 2000 through December 2002, and long-term average precipitation (line; Western Regional Climate Center 2003b).

moisture availability combined with cool temperatures to enhance germination by breaking dormancy. To examine *Gaura* seedling emergence under these conditions, we conducted a study using three pregermination treatments: cold-moist stratification for 61 days, 24-hr leaching, and 24-hr imbibition (control).

We also documented seedling emergence from capsules of seed lots differing in age and environment of seed production. Seed lots were obtained from field collections and one nursery-grown seed lot from the Bridger Plant Materials Center (BPMC) in Montana. The comparison of the older field-derived capsules to younger capsules allows us to evaluate viability of stored seeds within capsules and indirectly suggests the importance of the seed bank versus annual seed crop for recruitment. Comparison of the BPMC and WAFB collections allowed us to evaluate effects of maternal plant environment on capsule viability.

Increased knowledge of germination and dormancy-breaking mechanisms in *Gaura*, combined with the effects of vegetative manipulations upon recruitment (Munk et al. 2002; Burgess 2003), will aid development of management recommendations for sustaining or increasing *Gaura* populations. This study provides evidence of the factors that are most influential in stabilizing populations of this rare riparian forb species.

Materials And Methods

Site Description

Gaura seed capsules were collected from WAFB near Cheyenne, Laramie County, Wyoming (lat 51°09' N, long 104°52' W). WAFB lies on 2,367 ha, ranging in elevation from 1,840 to 1,950 m above sea level. Long-term annual

precipitation is 390 mm, although the region has been in a drought cycle with below-average rainfall since 1998 (Fig. 1; Western Regional Climate Center 2003b). Soils of *Gaura* habitat on WAFB include fine-loamy, mixed calcareous, mesic Fluvaquent Endoqualls of the Merden series and fine-loamy, mixed superactive, frigid Cumulic Endoqualls in the Kovich series (Stevenson 1997). We examined *Gaura* populations in three riparian sites at WAFB. Soils in the Diamond Creek and Unnamed Drainage are moist, sandy loams, whereas Crow Creek sites have well-drained, sandy gravels (Stevenson 1997). The BPMC irrigated nursery is located near the town of Bridger, in Carbon County, south-central Montana, at 1,127 m elevation. The long-term normal annual precipitation at BPMC is 345 mm (Western Regional Climate Center 2003a).

Seed Collection and Storage

Six seed lots were used in germination and viability trials. On 15 August 2001 we collected seed capsules from *Gaura* plants in three sites (Crow Creek, Diamond Creek, and Unnamed Drainage) at WAFB and stored them for 16 months (WAFB-16). In 2002 we collected capsules on 6 August (WAFB-4), 28 August (WAFB-3), and 3 October (WAFB-2) from the same three populations. Capsules collected earlier in the season (August) were darker green than those collected in October. Because there is little genetic variation among *Gaura* populations in the three riparian sites at WAFB (Brown 2000), seed lots from the three riparian sites at WAFB were combined by collection date prior to testing.

Two seed lots were obtained from the BPMC. One lot was collected in August 1998 from WAFB (WAFB-51) and stored at BPMC. The final seed lot was derived in 2001 from plants grown at the BPMC from the WAFB-51

seed lot (BPMC-16). All seed lots were stored in closed containers at room temperature from time of collection until initiation of the experiment.

Viability Testing

Because capsules do not naturally dehisce, and the capsules are quite woody and small, the seeds within them cannot be extracted without being damaged. For this reason, we used intact capsules for viability tests. Four *Gaura* seed lots (WAFB-51, WAFB-4, WAFB-16, and BPMC-16) were tested for capsule and seed viability using tetrazolium chloride. We randomly selected 150 capsules from each seed lot, scored the capsules on two sides to enhance imbibition (Voigt 1977; Peters 2000), and soaked them in a 1% tetrazolium chloride solution for 20 hr at room temperature (Cottrell 1947). Capsules are permeable to water, even when they are not scored, and can take up more than 50% of their mass in 24 hr (Burgess 2003). We subsequently dissected the capsules to document capsule viability (in this study capsules containing at least one viable seed) and seed viability (number of viable seeds in each capsule). Seeds in each capsule were observed under a microscope for firm texture, intact appearance, and uniform red staining. Total numbers of viable and nonviable seeds were recorded for each of the 150 capsules for three of the seed lots (WAFB-16 was evaluated only for capsule viability). Capsule and seed viabilities of the smaller WAFB-3 and WAFB-4 seed lots (end of production cycle, postfrost) were not tested, in order that adequate numbers of capsules could be retained for seedling emergence studies.

Seedling Emergence Study

Capsules from four seed lots (WAFB-16, WAFB-4, WAFB-3, and WAFB-2) were exposed to three pregermination treatments, (1) 2-month cool-moist stratification, (2) 24-hr leaching, and (3) 24-hr imbibition (control), to examine seedling emergence from capsules. The cool-moist stratification was initiated on 14 November 2002 using three replicates of 45 capsules from each seed lot. Capsules were placed in petri dishes lined with filter paper and filled with distilled water. Following 24 hr of imbibition excess water was drained, and on 15 November 2002 dishes were transferred to a refrigerator and held at 4°C for 61 days for cool-moist stratification. The filter paper substrate was rewetted as needed to maintain saturation.

A 24-hr leaching treatment was imposed on three replicates of 45 capsules of the four seed lots. We attached 15-L/hr (4 gph) drip irrigation emitters at 10-cm spacings to a 1.3-cm-diameter poly pipe greenhouse water source. Mesh bags containing each replicate of 45 seeds were attached to the emitters with rubber bands, and water was dripped through the bags for 24 hr on 14 January 2003. Mesh bags were very small (1-dm-diameter pouches) and were repeatedly examined to ensure that all capsules were

wetted. In addition, three replicates of 45 capsules from all six seed lots were also placed in filter paper-lined petri dishes and imbibed in distilled water for 24 hr beginning on 14 January 2003 to serve as controls for the other two pregermination treatments.

On 15 January 2003 all capsules from the three treatments were planted (3-mm depth to just cover capsules) in 4 × 4-cm plastic pots containing germination media (55% sphagnum peat moss, 25% perlite, and 20% vermiculite). We placed capsules in soils because the irregular shape of capsules seemed to limit their contact with wet filter paper, and emergence from capsules in prior growth chamber experiments on filter paper-lined petri dishes and in germination sleeves was very low. Germination media provided better contact of the capsules with moisture and improved overall seedling emergence. Pots were randomly arranged on benches in a greenhouse and randomized on the benches each week. All pots were watered daily. Ambient temperatures in the greenhouse ranged from 13°C (55°F) at night to 27°C (80°F) during the day. Day lengths ranged from 9.5 hr on 15 January 2003 to 12 hr on 18 March 2003 and were intended to match day lengths when this species is thought to germinate in the field setting.

We monitored seedlings that emerged from capsules on eight observation dates (29 January, 6 February, 12 February, 19 February, 26 February, 6 March, 10 March, and 18 March 2003) at about 1-week intervals (14, 22, 28, 35, 42, 50, 54, and 62 days after sowing). On each date we recorded the number of capsules with at least one emerged seedling and the number of seedlings per capsule. Viability tests were not conducted on capsules with no germinated seeds at the conclusion of the study because these capsules were difficult to distinguish from organic matter in the germination media after a few weeks of exposure to the moist conditions in the greenhouse.

Statistical Design and Analysis

The experimental design for the pregermination treatment study conducted on the four seed lots was a factorial arrangement of a completely randomized design with two factors (seed lot and pregermination treatment). Analysis of variance (ANOVA) was conducted appropriate to a completely randomized factorial design (SAS Institute 2000) using the sampling dates as repeated measures. Analysis of seedling emergence from 24-hr-imbibed capsules was used to assess all six seed lots, using a completely randomized design with observation date as repeated measures. In both seedling emergence studies, mean separation was conducted only when *F* ratios were significant at the 0.05 alpha level. Mean separations were conducted using Fisher's protected least significant differences. Because we anticipated changes in seedling emergence with time, we restricted our mean separation to comparison of seed lots within observation dates to limit multiple comparisons. Viability data are reported directly

Table 1. Characteristics of *Gaura* seed lots used in germination and viability studies.

Seed Lot (Origin & Age in Months) ^a	Date of Capsule Collection	Pregermination Treatment ^b	Seeds per Capsule (no.)	Viable Seeds per Capsule	Capsule Viability ^c (%)	Seed Viability (%)
WAFB-51	15 August 1998		2.4	1.9	81	79
WAFB-16	15 August 2001	X	na	na	61	na
BPMC-16	28 August 2001		1.9	1.4	73	70
WAFB-4	6 August 2002	X	1.0	1.0	54	63
WAFB-3	28 August 2002	X	X	na	na	na
WAFB-2	2 October 2002	X	X	na	na	na

^a na = not available. Age of seed collection is indicated in months, at time of seedling emergence experiments.

^b Indicates seed lots collected at WAFB in 2001 and 2002 that received a 2-month stratification and leaching treatments. All seed lots received a 24-hr imbibition treatment.

^c Capsule viability reflects capsules that contain at least one viable seed, based on tetrazolium chloride tests; $n = 150$.

as percentage of original capsule and seed numbers and were not subjected to ANOVA.

Results

The oldest seed lot in our collections, the WAFB-51 seed lot, contained more capsules with at least one viable seed and the most viable seeds per capsule of any of the seed lots we examined (Table 1). Viability of seed lots was not compared statistically.

After 61 days of cool-moist stratification, *Gaura* capsules were covered with black mold. Leached capsules were slightly discolored and pale yellow-green. Cool-moist

stratification-treated capsules had the greatest number of capsules with at least one seedling on all eight observation dates (observation date by treatment interaction, $p = 0.01$; Fig. 2). Leached capsules had lower seedling emergence (at least one seedling per capsule) than controls on only one observation date (28 days after sowing); germination in the leached and imbibed treatments was comparable on all other dates (Fig. 2). Seedling emergence did not differ among seed lots on any observation date ($p = 0.73$) or when averaged across all dates ($p = 0.54$).

Seedling emergence (at least one emerged seedling per capsule) from 24-hr-imbibed capsules did not differ among the six seed lots depending on date (seed lot by observation date interaction, $p > 0.05$). Observation dates

Figure 2. Proportion of *Gaura* capsules (%) with at least one emergent seedling under three pregermination treatments (61-day cool-moist stratification, 24-hr leaching, and 24-hr imbibition) 14–62 days after sowing. Error bars are provided only for comparison of the three pregermination means within an observation date (least significant difference = 0.64; $n = 45$).

Table 2. *Gaura* seedling emergence from capsules within six seed lots on eight observation dates.

Seed Lot	Days after Imbibition*							
	14	22	28	35	42	50	54	62
	<i>No. of Seedlings Emerged</i>							
BPMC-16	0.3	7.0	7.6	11.3	13.0	13.0	13.6	16.0
WAFB-51	0.0	4.0	4.6	9.3	10.6	11.0	11.0	13.0
WAFB-2	0.3	5.3	5.3	7.3	8.3	9.3	9.3	12.0
WAFB-3	0.0	0.3	1.0	4.0	5.6	6.0	8.3	8.3
WAFB-4	0.0	0.3	4.5	4.5	4.5	6.3	6.7	7.0
WAFB-16	0.7	1.7	2.8	3.7	4.3	4.3	5.0	5.3

(Values are the mean of three replicates of 45 *Gaura* capsules each.) * Capsules were imbibed in distilled water for 24 hr prior to placement onto germination media.

differed in number of seedlings emerged ($p < 0.01$; Table 2). On the first observation date 14 days after sowing, emergence was similar for all seed lots and increased over time. In general, seedling emergence from capsules was high in the horticulturally produced seed lot (BPMC-16). Capsules collected at WAFB during different months of 2002 suggests that as many seedlings emerge from later collections (October; WAFB-2) as from earlier collections (August; WAFB-4 and WAFB-3). Overall, the percentage of capsules with at least one seedling emerged from BPMC-16, WAFB-51, and WAFB-2 seed lots was 23, 17, and 16%, respectively. In other seed lots, less than 11% of the capsules imbibed had at least one seedling emerged.

Discussion

Our study is limited by the number of capsules available for us to collect from wild populations and by the fact that all capsules were stored in dry containers immediately after harvest. Environmental conditions experienced by the seed bank in situ are much different. Buried capsules are continually wetted and dried, and warmed and cooled; such field conditions most likely reduce seed viability, longevity, and germinability. However, because our oldest seed lot retained viability, it remains unclear how long *Gaura* can be stored before its viability declines. More mature collections made later within the same year appear to improve germination success, though not significantly. Coloration of capsules may indicate maturation of collections made later in the season (Baskin & Baskin 2001). Optimal timing of collection could be partially gauged by appearance of the seed capsules. Our data and observations suggest that young, dark green *Gaura* capsules are probably not fully mature and seed within immature capsules will have lower germination rates than seed within older, lighter green capsules. However, the observation that the nursery-produced seed lot (BPMC-16) exhibited relatively high germination probably reflects the favorable conditions within which maternal plants were grown. These results may also suggest that seeds are less

dormant when produced in a nursery, which could be a disadvantage if they germinate under inappropriate conditions when they are seeded in the wild. This finding may be especially consequential if attempts to stabilize *Gaura* populations are based on nursery-produced seed lots. Nursery plantings may favor genotypes with lower dormancy, and this unintentional restriction of germplasm could prove problematic for rare plant conservation in the natural setting.

Because cool-moist-stratified capsules had greater germination than leached and imbibed capsules, *Gaura* appears to have dormancy mechanisms associated with its northern latitude range. At WAFB average long-term normal daily minimum temperatures are at or below freezing for 5 months annually (Western Regional Climate Center 2003b). Combined chilling and moisture availability appear to be critical for *Gaura* germination. Species populations from habitats with severe winters can produce seeds that require long chilling treatments, whereas seeds of the same species produced in milder climates have shorter chilling requirements for germination (Meyer et al. 1995). Many species in the Onagraceae occur in wet sites in northern latitudes and so might be expected to require prechilling. On WAFB greater *Gaura* flowering has been documented 2 years after cool springs (Laursen & Heidel 2003).

There may be another dormancy mechanism inhibiting *Gaura* germination because it can take several weeks or months for some seedlings to emerge from capsules. In our study some of the discrepancy between viability and seedling emergence may be due to dormancy mechanisms not considered here. For example, some species develop mechanisms that prevent germination even when growing conditions are suitable; this has been interpreted as a mechanism that ensures the maintenance of a seed bank for future years (Meyer et al. 1995). Within the Onagraceae, five genera and 19 species have been found to have persistent seed banks (Baskin & Baskin 2001). Longevity of *Gaura* capsules under laboratory conditions suggests that capsules could persist in the soil, building the seed bank for several years until environmental conditions favor germination. In addition the reduced emergence of seedlings from more recently collected capsules relative to capsules

stored for 51 months may suggest that *Gaura* may have physical dormancy mechanisms that are broken by after-ripening. Cold treatments reduce physiological dormancy (Baskin & Baskin 2001) and because *Gaura* is a C3 species, this scenario seems reasonable. In addition, germination in other members of Onagraceae, most notably *Epilobium angustifolium* L., is improved with a 30-day stratification, and scarification of capsules was not needed (McLean 1967). However, it is still unclear if dormancy-breaking treatments result in changes in the embryo itself, are needed to impact the hard capsules, or both. Our stratification treatment resulted in disintegration of the capsule (mold was common), but seedling emergence was not improved when we scarified capsules with a sharp object (data not shown). In the field, extensive scarification as a result of mold, freeze-thaw, and tumbling over-hard substrate may facilitate degradation of the hard capsules. *Gaura* dormancy mechanisms require further examination.

Factors that are important for *Gaura* habitats may apply to other wetland restoration efforts. Keddy's (2000) list of important environmental factors that determine the properties of wetlands places hydrology as the most important factor, followed by fertility, salinity, disturbance, competition, grazing, and burial. Our results support hydrology as critical for *Gaura* recruitment. Historic changes have altered riparian corridors on WAFB. Early records show that the area was devoid of large trees until cottonwoods and other species were planted by residents (Barlow & Knight 1999). In grassland riparian systems of the Intermountain West, flooding keeps herbaceous meadows intact by limiting encroachment of shade-producing woody species. Beaver activity, which reduces woody materials and increases ponding behind dams, broadens the herbaceous meadows favorable to *Gaura*. Since this military base was established in 1867 (originally as a cavalry post), WAFB riparian corridors have been subjected to alterations in hydrology resulting from domestic water withdrawal, heavy grazing by domestic stock, military activities, residential development, and managed stream flows. Beaver removal at WAFB has allowed willow encroachment and reduced the breadth of herbaceous meadows and suitable *Gaura* habitats there.

Because exposure to cool temperatures and extended periods of moisture availability enhance *Gaura* germination, altered hydrology of *Gaura* habitats on WAFB may be an important contributor to the species' decline. *Gaura* requires bare substrate and little shade for juvenile recruitment (Floyd 1995; Fertig 2000) and exposure of capsules to cool-moist conditions for extended periods to enhance germination. Reduced beaver activity has likely contributed to the reduction in *Gaura* recruitment success. Future study is needed to test this assertion, and in situ work on *Gaura* seed banks is also needed to understand capsule decay and seed longevity in natural settings. When coupled with surface vegetative manipulation studies currently under way, it may be possible to develop manage-

ment techniques that stabilize self-sustaining populations of this rare herbaceous species.

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