Long-Term Storage of Riparian Salicaceae Seed for Direct Seeding Applications: Analysis of Seed Germination and Vigor

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Abstract:

On the lower Colorado River, re-vegetation of Fremont cottonwood (*Populus fremontii* S. Watson [Salicaceae]), Goodding's willow (*Salix gooddingii* C.R. Ball [Salicaceae]), and coyote willow (*S. exigua* Nutt. [Salicaceae]) is planned to partially restore riparian ecosystems degraded or removed by land use conversion and river management practices. Currently, vegetative propagation is used for revegetation. If direct seeding could be implemented, genetic and structural diversity could be enhanced at restoration sites even while reducing costs compared to vegetative propagation methods. Previous observations indicate that seed viability for these species might be maintained for less than 1 month, which might limit feasibility for direct seeding. We implemented germination tests to examine if experimental storage conditions might extend periods of seed viability. Storage at 21^{o}C (70^{o}F) resulted in decreased germination rates after 2 months for all species. Seed stored at freezing temperatures maintained germination rates of greater than 80% for more than 2 years. Cleaning or storage without oxygen did not result in increased germination rates after 2 years of storage in freezers. Following 1 year of storage in freezers, similar vigor was found for seed collected 2 months prior to seeding and seed stored in freezers for 14 months. These results indicate that seed viability can be extended by freezing to at least a 2-year window during which seed can be utilized either for direct seeding or production of seedlings for outplanting.

Key Words: cottonwood; *Populus*; re-vegetation; riparian; Salicaceae; *Salix*; willow.
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Introduction

River systems throughout the world have been dramatically altered for anthropogenic uses. As a result of river flow management and land development within historic floodplains, wetlands along major rivers have been dramatically reduced. Following realization of the large benefits of wetlands, including flood control and habitat for native fauna (Mitsch and Gosselink 2000), restoration of wetlands has become a major objective of various land managers throughout the world. Along the lower Colorado River (LCR), the portion of the Colorado River between Lake Mead and the Colorado River Delta, areas of riparian vegetation have historically been cleared to allow for agricultural land use. During the 20th century, dams were constructed on the Colorado River from Lake Powell to the international border with Mexico. This flow regulation, along with clearing of native vegetation and establishment of non-native species, has resulted in soil salinization (Glenn et al. 1998), channel narrowing and incision (Shafroth et al. 2002), and an increase in the frequency and intensity of wildfires (Busch 1995). Native riparian trees have lower salinity tolerance (Glenn et al. 1998), lower fire tolerance (Busch 1995), and require shallower groundwater (Lite and Stromberg 2005) compared to exotic invasive species in the area. Therefore, historic ecosystem alterations have resulted in large-scale loss or degradation of native riparian communities. To mitigate degradation of riparian ecosystems on the LCR due to current and future river management practices, the U.S. Bureau of Reclamation plans to re-vegetate 2,400 ha (6,000 ac) of land previously under agricultural use or dominated by saltcedar (*Tamarix ramosissima* Ledeb. [Tamaricaceae]), an introduced invasive species, with the native Salicaceae species Fremont cottonwood, Goodding's willow, and coyote willow to provide habitat for native fauna (Bureau of Reclamation 2006).

Current Riparian Habitat Restoration Practices and the Potential for Seedlings

For restoration projects, cottonwood, willow, and other Salicaceae species are typically established by vegetative propagation. Propagation methods include pole planting or placement of rooted or bare cuttings collected from source trees. Typically, pole planting is implemented by inserting branches into the soil to a depth within groundwater or the capillary fringe to prevent water stress (FISRWG 1998). On the LCR, the availability of long-term irrigation allows the U.S. Bureau of reclamation to outplant cuttings even where the depth to groundwater is relatively high. Since 2005 the U.S. Bureau of Reclamation has been propagating small

cuttings for this purpose. Approximately 10-cm (4 in) cuttings are grown for a period of up to eight months within nurseries to establish roots, and then outplanted at very high densities (up to 1.7 m^{-2} [0.16 ft^{-2}]) at restoration areas using standard farming equipment (Bureau of Reclamation 2007).

Despite the high success of small cuttings in restoration areas, there are concerns with using vegetative propagation for large-scale revegetation, particularly for dioeceous species such as those of the Salicaceae family (Landis et al. 2006). Stems are often taken from a limited number of sources trees, which affects genetic diversity, growth rates, and sex ratios at restoration sites (Winfield and Hughes 2002). In general, contractors pay little attention to the sex or number of source trees, jeopardizing the likelihood of self-sustaining plant communities at restoration sites (Landis et al. 2003). Additionally, vegetative propagation results in high costs due to the need for collection, storage, transportation, and preliminary establishment in controlled environment agricultural systems (Bureau of Reclamation 2007).

High-density establishment of seedlings has been observed in riparian systems which still experience seasonal flooding and scour. For example, dense establishment of Fremont cottonwood and Goodding's willow has been observed along the Bill Williams River, a tributary of the LCR which has experienced seasonal flooding during the past decade (Shafroth et al. 1998). Figure 1 shows a sandbar on the Bill Williams River colonized by Fremont cottonwood and Goodding's willow following natural seedfall. Natural recruitment of native Salicaceae trees has also been observed following flooding in the Colorado River Delta (Nagler et al. 2005) and on the middle Rio Grande in New Mexico (Taylor and McDaniel 1998, Taylor et al. 1999, Sher et al. 2002, Sprenger 2002). In Colorado, drawdown of ponds within gravel pits has been choreographed during seed dispersal to promote germination and seedling growth (Roelle and Gladwin 1999, Roelle et al. 2001). Timed irrigation where soil is not inherently moist would likely encourage regeneration where native tree seedfall is abundant. However, where natural seedfall is lacking due to reduced local populations, supplemental seed application might be required. Although wild seed collection opportunities might be limited along other rivers in Arizona (Dreesen 2003), remnant Salicaceae communities and established orchards on the LCR allow for efficient field seed collection.

Direct seeding typically reduces revegetation costs compared to outplanting of propagated stock or seedlings. For example, Schuman et al. (2005) reported a reduction of over 90% in the cost of native shrubs per plant by direct seeding compared to hand-planting seedlings (other discussions of cost reductions due to direct seeding for agriculture or revegetation include Willoughby et al. (2004), Dissanayake et al. (2008), and Balandier et al. (2009)). Therefore, it is likely that, if direct seeding can be implemented for Salicaceae species, the costs of large-scale riparian habitat restoration could be dramatically reduced.

Salicaceae Seed Viability

The literature generally describes viability of cottonwood and willow seed as one to several weeks under field conditions (Stromberg 1993, Young and Clements 2003, Daigle and Simpson 2009), and it has therefore been suggested that harvested seeds must sown immediately after collection (e.g. Landis et al. 2006). Very short periods of seed viability could preclude direct seeding because of the narrow window available for seed collection and treatment, site preparation, seeding, and irrigation. However, studies are lacking which address the potential for long-term preservation of harvested seed. Storage of several *Salix* spp. (*S. bebbiana* Sarg., *S. discolor* Muhl., and *S. eriocephala* Michx. [Salicaceae]) at sub-freezing temperatures indicated that high germination rates can be maintained for several years (Simpson and Daigle 2009). Such studies have not been implemented for species specific to the LCR. To address this knowledge gap, a study was implemented to analyze the effectiveness of various seed preservation techniques in extending the viability of Fremont cottonwood, Goodding's willow, and coyote willow seed for a 2-year period. This time period would allow for flexibility in the implementation of large-scale seeding. Additionally, a greenhouse study was conducted to determine if long-term storage adversely affects seed vigor and resultant establishment and growth.

Salicaceae seed is highly pubescent, as shown for Fremont cottonwood in Figure 2. Removing pubescence from seed might reduce translocation by wind and water following seeding and reduce the storage space needed. Potential cleaning methods include blowing pubescent seed through sieves (Dreesen 2003) or vacuuming through screen (Dawes 2003). Preliminary

analyses during this study indicated that seed cleaning would reduce the required storage space by more than 90%. However, it has been suggested that hair removal could detrimentally affect germination rates (Sher and Marshall 2003). Additionally, the labor required for seed cleaning might offset the economic gains of a reduction in required freezer space. Other seed storage conditions to consider include oxygen concentration and storage temperatures. Oxygen reduction in hermetic containers and freezing of seed have been shown to increase or extend seed viability in a variety of species because these conditions reduce seed metabolism (Justice and Bass 1978).

A common observation for agricultural crops is that seedlings exhibit variable growth rates (vigor) as a function of seed storage duration and conditions (Delouche and Caldwell 1960). Even if Salicaceae seed viability can be maintained for extended periods, it might be possible that direct seeding would fail due to reduced growth rates of seedlings and intense competition with volunteer species for sunlight and soil moisture.

Study Methods

Seed germination and seedling growth studies were implemented with Fremont cottonwood, Goodding's willow, and coyote willow seed collected from native and planted trees on the LCR. Seeds were passively dried in the laboratory, and then stored either cleaned or un-cleaned, under ambient or freezing temperatures, and ambient oxygen or oxygen-free conditions (Table 1). Periodic germination tests were conducted in incubators to determine trends over time and the effectiveness of storage treatments. A greenhouse seeding study was also implemented to determine if seed stored for long periods might exhibit similar establishment and growth rates as newly-collected seed.

Seed treatments which might ease seed storage requirements, increase establishment rates, and/or extend the viability of collected seed included: the removal of seed hairs; removal of oxygen from storage containers; and storage in freezers. The study analyzed all combinations of treatments, resulting in a factorial study design: Seed Cleaning × Oxygen Condition × Storage Temperature.

Seed Collection Methods

Fremont cottonwood and Goodding's willow seed was collected from Cibola National Wildlife Refuge (NWR) in Cibola, Arizona, and coyote willow seed was collected from the Ahakhav Tribal Preserve in Parker, Arizona. Because the timing of seed collection is critical to obtaining mature, viable seed (Daigle and Simpson 2009), catkins were collected from source trees (two for each species) which had begun actively-dispersing seed. Mature Fremont cottonwood seed was generally observed to be tan when mature. Goodding's willow seed appeared black to the naked eye, and Coyote willow seed appeared dark green. Fremont cottonwood seed was collected by removing seeding branches with a pruning pole. Goodding's and coyote willow seed was collected by pruning catkin-laden branches or picking catkins from the source trees by hand. Plant waste was trimmed and catkins were stored in paper bags for transport to the University of Arizona Southwest Center for Natural Products Research and Commercialization (NPC, Tucson, Arizona). To promote capsule drying and dehiscence, seed from each paper bag was split into several bags and placed on laboratory benches for a period of one week.

Seed Treatment Methods

To remove seed pubescence, seeds were blown through a series of soil sieves (0.85 mm, 1 mm, 0.5 mm, Newark Wire Cloth Company, Clifton, NJ) using compressed air as described by Dreesen (2003). Cleaned seed was collected on the bottom screen and placed into storage treatment the same day as cleaning. Seed stored in ambient air conditions was placed either in paper envelopes (room temperature [21^{o}C (70^{o}F) storage) or plastic bags (frozen storage). Frozen storage was at -19^{o}C (-2.2^{o}F) for Fremont cottonwood, and at -10^{o}C (14^{o}F) for Goodding's and coyote willow per recommendations from the US Department of Agriculture National Seed Storage Laboratory (Didericksen, Biological Science Laboratory Technician, January 9, 2005, personal communication). Seed subject to oxygen removal was placed into glass vials with a septum cap (VW60810-1232 vial with VW73804A8425 septum cap, VWR International, West Chester, PA), and 3 cycles of air evacuation and nitrogen gas injection were implemented. Enough seed was placed in each vial, envelope, or plastic bag for 1 germination test.

Germination Rate Determination

Seeds from each source tree and treatment were placed between moist paper towels and placed in an incubator (VWR Economy Incubator CSA1500E, VWR International, West Chester, PA). For each test, a minimum of 5 seeds were sown per source tree, with an average of 18 seeds per source tree; a minimum of 11 seeds total per species was tested, with an average of 35 seeds for each species per test. De-ionized water was sprayed daily on each paper towel to maintain an optimum environment for germination. To maintain moisture for willow seeds, which are much smaller than those of Fremont cottonwood, paper towels with these species were placed within plastic Petri dishes. One incubator was set at 19^{o}C (66^{o}F) for Fremont cottonwood, and one set at 27^{o}C (81^{o}F) for both willow species, to provide optimal germination temperatures (Baskin and Baskin 1998). After a minimum of 3 days, seeds were observed beneath a magnifying glass for the emergence of cotyledons which indicated a positive result.

Following air drying on laboratory benches, and prior to initiation of cleaning, freezing, or oxygen removal treatments, an initial germination test was conducted. Following implementation of storage treatments, bi-weekly germination tests were planned. However, due to maintained viability, the time between germination trials was increased so that germination trials could be conducted for 2 years given the number of seed containers in storage. Due to low germination rates for room temperature storage after 19 weeks, testing of seed under this treatment was discontinued thereafter.

Greenhouse Test Pot Study

To determine the potential for variability in seed vigor following 1 year of storage, a randomized block 26.5 l (7 gal) test pot study was implemented at the NPC to test the effects of seed storage and seed cleaning on tree establishment and growth in soil excavated from an agricultural field on the LCR (Cibola National Wildlife Refuge, Cibola, Arizona). In addition, an organic fertilizer was added to half of the pots to determine if fertilizer amendments would increase plant growth rates, and therefore overcome potential decreases in seed vigor that might occur following long-term storage.

A total seeding rate of 645 pure live seeds (PLS) m^{-2} (60 ft^{-2}), approximately 215 PLS m^{-2} (20 ft^{-2}) each of Fremont cottonwood, Goodding's willow, and coyote willow was applied. This seeding rate was previously observed to result in high establishment and growth of all 3 species when seeded together (GeoSystems Analysis, Inc. 2007). Biosol (Biosol Organic Fertilizers, Denver, CO) was added at a rate of either 0 Mg ha^{-1} (0 $lb.ac.^{-1}$) or 1.68 Mg ha^{-1} (1,500 $lb.ac.^{-1}$), as suggested for application on disturbed soils (Tom Bowman 2006, Rocky Mountain Bio Products, Denver, Colorado, personal communication) and as observed during previous studies to increase growth rates of seeded Salicaceae species in nutrient-deficient soils (GeoSystems Analysis, Inc. 2007). Biosol consists of sterilized *Pennicilium* fungal biomass with added Nitrogen (as percent N), Phosphorus (as percent P_2O_5), and Potassium (as percent K_2O) at 7-2-3.

The greenhouse study analyzed all combinations of treatments in triplicate, with additional consideration for placement within the greenhouse (i.e. 1 replicate for each treatment combination was placed within greenhouse block 1, 2, and 3). The resulting study treatment matrix was Seed Collection Year × Seed Type × Organic Fertilizer + Greenhouse Placement (Table 2).

Cottonwood and willow seed was collected from native and planted trees during April 2006, and March and April 2007. The seed was stored un-cleaned in freezers under ambient oxygen conditions until the day of planting. PLS rates were determined from incubator germination results collected the previous week (seed tested prior to seed cleaning). The analysis determined the appropriate number of seeds to place in each pot such that the resulting pure live seed rates were similar for year-old and freshly collected seed. On the day of planting, seed was removed from freezers. Seed for the cleaned seed treatments were cleaned in a Wiley mill (Model #2 and Model #4, Arthur H. Thomas Company, Philadelphia, PA) with subsequent separation of seed from debris with a #25 sieve (Newark Wire Cloth Company, Newark, NJ). Final cleaning was accomplished with an air-screen machine (Model D, E.L. Erickson Products, Brookings, South Dakota). Seed for each pot was counted by hand and placed in an envelope labeled with the appropriate pot number.

Planting took place on June 1, 2007, during the typical period of seed dispersal on the LCR, and irrigation was initiated immediately. Thereafter, test pots were irrigated twice per day until ponding was observed. Between October 3 and October 8, 2007, all trees were clipped from pots at the soil surface, counted, individually measured for height, dried in a heated drying room, and weighed. A linear analysis of variance (ANOVA) was used to determine the effects of implemented treatments on establishment and growth rates for one growing season.

Results

Seed Storage Analysis

Figure 3 shows that, except for un-cleaned seed with oxygen removed, germination rates declined for both cleaned and un-cleaned Fremont cottonwood seed stored at room temperature between 8 and 12 weeks. Oxygen removal extended favorable germination rates for un-cleaned seed at room temperature from 7 and 17 weeks for this species. Results in Figure 4 show that freezing seed generally resulted in germination rates of over 80% throughout the 2-year study period. Seed cleaning generally did not result in differences in germination rates. However, oxygen removal adversely affected cleaned seed under both frozen (germination rates as low as 43% at end of study) and room temperature storage (no germination after 3 weeks of storage). Oxygen removal did not result in increased germination rates for any of the un-cleaned, frozen Fremont cottonwood seed germination tests.

Figure 5 shows that germination rates of cleaned and un-cleaned seed stored at room temperature declined to 0% between 8 and 12 weeks for coyote willow, whereas Figure 6 shows that freezing seed resulted in germination rates of over 80% for the two-year study period. Oxygen removal did not extend the viability of seed stored at freezing or room temperatures during any of the coyote willow germination trials. Seed cleaning did not generally result in differences in germination rates.

Figure 7 shows that germination rates of cleaned and un-cleaned Goodding's willow seed stored at room temperature declined between 8 and 12 weeks. Oxygen removal increased germination rates of un-cleaned seed stored at room temperature until 15 weeks, but no germination was observed after 17 weeks. Figure 8 shows that freezing of seed generally resulted in germination

rates of over 80% for the entire two-year study period for this species. Cleaning generally did not result in germination rate differences. Oxygen removal did not result in increased germination for frozen Goodding's willow during any of the germination trials.

Seed germination results for all species and treatments are shown in Table 3 after approximately 1 and 2 years of storage, which represent the times from seed collection to when direct seeding would most likely be implemented. Seed viability for ambient temperature storage was assumed as 0 for all species after one year. Germination rates of over 80% were observed for frozen Fremont cottonwood, Goodding's willow, and coyote willow both 1 and 2 years after seed collection. Oxygen removal did not increase germination rates of any species for these tests (at P=0.05). Cleaning of Fremont cottonwood seed resulted in decreased germination rates, whereas cleaning of Goodding's and coyote willow seed did not affect germination rates.

Test Pot Study

ANOVA results for the pot studies are provided in Table 4. The interaction of seed cleaning by seed storage for Goodding's willow is shown in Table 5. Seed cleaning resulted in increased tree establishment and biomass of Fremont cottonwood and coyote willow compared to un-cleaned seed by up to 300%. With the exception of cleaned, 14-month old Goodding's willow seed, seed storage duration did not result in decreased tree establishment or plant growth at P=0.05 (dry plant biomass) (Table 4). Significant reductions in tree establishment (stem counts) and biomass production were observed for cleaned year-old Goodding's willow seed compared to the fresh seed and un-cleaned seed. Cleaned fresh Goodding's willow seed resulted in higher stem counts compared to un-cleaned seed from the same collection. Un-cleaned year-old Goodding's willow seed resulted in higher dry biomass compared to fresh un-cleaned seed. Establishment was similar for un-cleaned seed between years (Table 5). Biosol addition did not result in increased growth rates and reduced riparian tree establishment. In fact, biomass was reduced in organic amended test pots due to decreased stem counts (Table 4).

Conclusions and Discussion

Germination of Fremont cottonwood, Goodding's willow, and coyote willow seed stored at room temperature was limited to less than 2 months. However, storage of seed at -19^{o}C for Fremont

cottonwood, and at -10^{o}C for Goodding's and Coyote willow resulted in high germination rates for over 2 years. Additionally, seed stored for 1 year exhibited similar vigor to freshly-collected seed. These results indicate that seed viability does not preclude the feasibility of direct seedling Fremont cottonwood, Goodding's willow, or coyote willow for large-scale revegetation. If seeding can be coordinated to occur within a few weeks of seed collection, it is likely that seed can be stored at room temperature. If an extended time period (greater than 1 month) will pass between seed collection and seeding, seed should be stored in freezers.

No benefits to germination rates were shown from seed cleaning. Therefore, seed cleaning prior to storage is only useful to reduce storage space and freezer constraints and costs. If storage space is not a constraint, seed can be stored un-cleaned. Although un-cleaned storage would increase storage costs, it would reduce seed processing labor costs. Greenhouse studies of broadcast-seeding these Salicaceae species have indicated that seed cleaning might result in an approximate doubling of established seedlings (GeoSystems, Analysis, Inc. 2007). Therefore, less seed would be required for a given target tree density given broadcast seeding. The reduced amount of seed required to be collected and processed would offset at least a portion of the increased costs accrued during seed storage.

Suitable large-scale seeding methods might also dictate the necessity for seed cleaning. For example, seed cleaning would be required for broadcast seeding or drill seeding, whereas either cleaned or un-cleaned seed can be applied with hydroseeding equipment. Preliminary, small-scale field trials have indicated that hydroseeding of un-cleaned seed might result in establishment rates equal to or exceeding establishment rates of broadcast cleaned seed (GeoSystems Analysis, Inc. 2008). Therefore, a higher number of un-cleaned seeds might not be required for a given area of seeding. However, higher volumes of seed would still need to be stored in freezers compared to cleaned seed.

Oxygen removal from storage containers did not result in higher germination rates during this study. In fact, oxygen removal occasionally resulted in decreased germination. Because no benefit was observed and additional costs would be realized due to the need for specialized

containers and additional processing effort, oxygen removal is not recommended for seed storage for these species.

Plant establishment and growth rates were not significantly reduced after 1 year of storage in freezers. Therefore, it is likely that seed can be stored in freezers for at least 1 year prior to direct seeding. Seed cleaning after 1 year of storage in freezers resulted in reductions in the establishment of Goodding's willow, likely due to heat exposure during cleaning. Consequently, seed cleaning is recommended to occur soon after collection. Additionally, seed viability should be confirmed with germination testing after seed is cleaned, and soon before seeding is implemented.

Addition of organic fertilizer (Biosol) did not increase growth rates in seeded pots. Also, Biosol resulted in decreased establishment, likely due to fertilizer burning or elevated fungal growth on the soil surface. Therefore, this organic fertilizer is not required for seedlings established via seed stored for extended periods. However, it is of note that Biosol has been shown to increase the biomass of Salicaceae species seeded on sandier soils (GeoSystems Analysis, Inc. 2007). Therefore, the need for fertilizers should be assessed for site-specific soil texture and nutrient levels to determine if a reduction in tree establishment is an acceptable tradeoff for potentially higher tree growth rates.

Longer-term analyses of seedling growth rates (after several years of frozen storage) should be implemented to confirm that seed vigor is maintained. Additional studies are recommended for Salicaceae species desired for restoration in other regions. For example, it is likely that plains cottonwood (*P. deltoides* Bartram ex Marsh. [Salicaceae]) and Quaking aspen (*P. tremuloides* Michx. [Salicaceae]) can be similarly stored while maintaining seed viability and vigor. Preliminary investigations for Quaking aspen have indicated that stock might be produced from harvested seed, a positive result for a species which typically does not propagate well from stem cuttings (Landis et al. 2006).

Additional seed storage research for these species should include determination of seed moisture conditions on long-term viability. In general, optimal storage temperatures might depend on

moisture content (Justice and Bass 1978). However, storage of several *Salix* spp. at relative humidity between 5 and 10% was not observed to cause changes in viability for at least 5 years (Simpson and Daigle 2009).

To maximize the genetic diversity and maintain favorable sex ratios at restoration sites, it has been suggested that sexual propagation should be used whenever possible (Landis et al. 2003). In general, re-vegetation of Salicaceae species has been implemented via vegetative propagation due to perceived limitations of long-term seed viability. However, this study and similar work by Simpson and Daigle (2009) indicate that Salicaceae seed can be stored in freezing temperatures for extended periods of time, thereby allowing the use of either direct seeding or seedling propagation and outplanting for the enhancement of riparian restoration.

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Tables

Table 1. Seed storage treatments implemented for Fremont cottonwood, Goodding's willow, and coyote willow.

Variable	Treatment	Specifications
Seed Cleaning	Cleaned	Seed removed from catkins, blown through a sieve series using compressed air to remove pubescence.
	Un-Cleaned	Seed removed from catkins, stored with pubescence still attached.
Oxygen Condition	Oxygen Purged	Oxygen removed via air purging with a vacuum and replacement with nitrogen gas. Seed stored in glass vials.
	Ambient	Seed stored at ambient oxygen. Seed stored in envelopes (ambient temperature) or plastic bags (frozen).
Storage Temperature	Frozen	Seed stored in freezers at -10^{o}C (Goodding's willow and coyote willow) or -19^{o}C (Fremont cottonwood).
	Ambient	Seed stored in the laboratory at an average temperature of 21^{o}C (thermostat-controlled).

Table 2. Salicaceae 7-gallon pot study specifications.

Variable	Treatment	Specifications
Seed Collection Year	2007	Seed collected during March and April 2007 (approximately two months before seeding).
	2006	Seed collected during March and April 2006 (approximately 14 months before seeding).
Seed Type	Cleaned	Pubescence removed from seed coats using Wiley Mill.
	Un-cleaned	Pubescence not removed from seed coats.
Organic Fertilizer	0 Mg ha^{-1}	Biosol organic fertilizer not applied.
	1.68 Mg ha^{-1}	Biosol organic fertilizer applied at a nominal rate of 1.68 Mg ha^{-1}.
Greenhouse Placement	Block 1	Repetition 1, closest to evaporative cooling pads.
	Block 2	Repetition 2, middle of the greenhouse.
	Block 3	Repetition 3, furthest from evaporative cooling pads.

Table 3. Incubator-determined germination rates for Fremont cottonwood, coyote willow, and Goodding's willow seed stored in freezers at two time intervals after seed collection.

Species	Seeding Date	6/4/2007	7/9/2008
	Weeks of Storage	58	115
	Treatment	% Seed Germination (n)*	
Fremont Cottonwood	Cleaned, Oxygen Purged	76 (83) C	43 (67) C
	Cleaned, Ambient Oxygen	88 (76) B	87 (30) B
	Un-Cleaned, Oxygen Purged	98 (112) A	97 (61) A
	Un-Cleaned, Ambient Oxygen	100 (75) A	95 (61) AB
Coyote Willow	Cleaned, Oxygen Purged	87 (60) B	90 (67) A
	Cleaned, Ambient Oxygen	85 (54) B	91 (65) A
	Un-Cleaned, Oxygen Purged	80 (49) B	81 (63) A
	Un-Cleaned, Ambient Oxygen	97 (73) A	89 (64) A
Goodding's Willow	Cleaned, Oxygen Purged	92 (64) A	92 (39) A
	Cleaned, Ambient Oxygen	85 (73) A	87 (67) A
	Un-Cleaned, Oxygen Purged	83 (59) A	86 (64) A
	Un-Cleaned, Ambient Oxygen	85 (65) A	80 (15) A

* Letters indicate significant differences between storage treatments (within species only) at α=0.05.

Table 4. Linear ANOVA results for test pot study of fourteen-month old frozen Salicaceae tree seed compared to freshly-collected (two-month old). Tests were run using JMP V7.0.1 (SAS Institute, Inc., Cary, North Carolina).

Results	Stems m^{-2}			Dry Above-Ground Biomass, g m^{-2}		
	Fremont Cottonwood	Goodding's Willow	Coyote Willow	Fremont Cottonwood	Goodding's Willow	Coyote Willow
Main Effects	**p Values**					
Seed Collection Year	0.191	<0.0001	0.092	0.677	0.812	0.846
Seed Type	<0.0001	0.04	0.0002	<.0001	0.02	0.008
Organic Fertilizer	<0.0001	<0.0001	<0.0001	0.014	0.009	0.029
Greenhouse Block	0.928	0.414	0.589	0.24	0.186	0.506
Interactions	**p Values**					
Seed Type*Seed Collection Year	0.423	<0.0001	0.047	0.35	0.005	0.248
Seed Type*Organic Fertilizer	0.002	0.04	0.024	0.324	0.621	0.589
Organic Fertilizer*Seed Collection Year	0.787	<0.0001	0.092	0.833	0.216	0.986
Seed Type*Organic Fertilizer*Seed Collection Year	1	0.0005	0.047	0.717	0.275	0.189
Seed Collection Year	**Means and Significant Differences***					
2007	96.9 A	58.3 A	30.5 A	132.5 A	11.09 A	2.35 A
2006	78.9 A	27.8 B	48.4 A	144.8 A	10.29 A	2.17 A
Seed Type						
Cleaned	139.9 A	36.8 B	64.6 A	219.8 A	6.26 B	3.67 A
Un-cleaned	35.8 B	49.3 A	14.3 B	57.5 B	15.18 A	0.85 B
Organic Fertilizer						
0 Mg ha^{-1}	125.6 A	68.1 A	66.4 A	179.1 A	15.82 A	3.37 A
1.68 Mg ha^{-1}	50.3 B	18 B	12.6 B	98.2 B	5.56 A	1.16 B
Greenhouse Placement						
Block 1	91.5 A	45.7 A	32.3 A	105.5 A	15.39 A	2.15 A
Block 2	86.1 A	45.7 A	41.8 A	142.4 A	9.13 A	1.66 A
Block 3	86.1 A	37.7 A	44.5 A	168.0 A	7.67 A	2.97 A

*Numbers denote least-squared means, letters denote significant differences at p=0.05 within each main effect column according to Least-squared Means Differences Student's t-test.

Table 5. Linear ANOVA results for seed type × seed collection year interactions for greenhouse study of previous year (fourteen-month old), frozen Goodding's willow tree seed compared to freshly-collected (two-month old) seed. Tests were run using JMP V7.0.1 (SAS Institute, Inc., Cary, North Carolina).

Seed Type	Seed Collection Year	Goodding's Willow Stems m^{-2} *	Dry Above-Ground Biomass, g m^{-2}
Un-Cleaned	2006	53.8 B	20.5 A
Un-Cleaned	2007	44.8 B	9.87 BC
Cleaned	2006	1.79 C	0.14 C
Cleaned	2007	71.8 A	12.4 AB

*Numbers denote least-squared means, letters denote significant differences at p=0.05 within each main effect column according to Least-squared Means Differences Student's t-test.

Figures

Figure 1. Goodding's willow and Fremont cottonwood seedlings established on a sandbar along the Bill Williams River, Bill Williams River National Wildlife Refuge, La Paz County, Arizona. Photo by Matthew R. Grabau.

Figure 2. Pubescent Fremont cottonwood seed following dehiscence of capsules in a paper bag. Photo by Matthew R. Grabau.

Figure 3. Fremont cottonwood seed germination versus time for room-temperature storage treatments. Treatment codes are as follows: 1) laboratory temperature, cleaned, oxygen purged; 2) laboratory temperature, cleaned, ambient oxygen; 3) laboratory temperature, un-cleaned, oxygen purged; 4) laboratory temperature, un-cleaned, ambient oxygen.

Figure 4. Fremont cottonwood seed germination versus time for freezer storage treatments. Treatment codes are as follows: 5) -19^{o}C, cleaned, oxygen purged; 6) -19^{o}C, cleaned, ambient oxygen; 7) -19^{o}C, un-cleaned, oxygen purged; 8) -19^{o}C, un-cleaned, ambient oxygen.

Figure 5. Coyote willow seed germination versus time for room-temperature storage treatments. Treatment codes are as follows: 1) laboratory temperature, cleaned, oxygen purged; 2) laboratory temperature, cleaned, ambient oxygen; 3) laboratory temperature, un-cleaned, oxygen purged; 4) laboratory temperature, un-cleaned, ambient oxygen.

Figure 6. Coyote willow seed germination versus time for freezer storage treatments. Treatment codes are as follows: 5) -10^{o}C, cleaned, oxygen purged; 6) -10^{o}C, cleaned, ambient oxygen; 7) -10^{o}C, un-cleaned, oxygen purged; 8) -10^{o}C, un-cleaned, ambient oxygen.

Figure 7. Goodding’s willow seed germination versus time for room-temperature storage treatments. Treatment codes are as follows: 1) laboratory temperature, cleaned, oxygen purged; 2) laboratory temperature, cleaned, ambient oxygen; 3) laboratory temperature, un-cleaned, oxygen purged; 4) laboratory temperature, un-cleaned, ambient oxygen.

Figure 8. Goodding’s willow seed germination versus time for freezer storage treatments. Treatment codes are as follows: 5) -10^{o}C, cleaned, oxygen purged; 6) -10^{o}C, cleaned, ambient oxygen; 7) -10^{o}C, un-cleaned, oxygen purged; 8) -10^{o}C, un-cleaned, ambient oxygen.