

Old male catkins, like these on *Salix exigua* from the Hopi Reservation in Arizona, can be used to determine the sex of dioecious species during winter dormancy, and ensure that branch collections include both sexes.

Photo by Thomas D Landis

Thomas D Landis, David R Dreesen, Jeremy R Pinto, and R Kasten Dumroese

ABSTRACT

The USDA Forest Service, USDA Natural Resources Conservation Service (NRCS), and the Hopi Tribe Office of Range Management have been working together on native plant restoration projects in northeastern Arizona. The aggressive exotic plants, Russian-olive (*Elaeagnus angustifolia* L. [Elaeagnaceae]) and salt-cedar (*Tamarix ramosissima* Ledeb. [Tamaicaceae]), have invaded many wetland and riparian areas on the Hopi Reservation, excluding willows (*Salix* L.), cottonwoods (*Populus* L.), and other native plants. The tribe has been mechanically removing the invasives and has asked for help in propagating native species to plant in these project areas. Although much information is available on how to collect willows and cottonwoods and propagate them, some unique challenges exist on Hopi lands. Some species are common, while others are very rare and in some cases only a few individual plants exist. The scattered locations of streams, wetlands, and seeps must be considered during plant material collections to ensure that both genetic and sexual diversity are adequately represented. Another challenge is the determination of target plant stock types that are appropriate on the diverse hydrologic conditions on the various project sites. Collected plant materials were taken to the NRCS Plant Materials Center in Los Lunas, New Mexico, for both seed and vegetative propagation.

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KEY WORDS

restoration, culturally significant plants, invasive species, Intertribal Nursery Council, *Salix, Populus*

NOMENCLATURE

USDA NRCS (2005)

The Hopi Reservation is located in northeast Arizona (Figure 1A) where the tribe has been working to eradicate exotic salt-cedar (Tamarix ramosissima Leneb. [Tamaricacae]) and Russian-olive (Elaeagnus angustifolia L. [Elaeagnaceae]) from streams and wetlands. Comprising about 2% of the reservation, these riparian and wetland communities are ecologically and culturally valuable for livestock grazing, wildlife habitat, traditional gathering, use (Lomadafkie and ceremonial 2003). Although the initial eradications were successful, the saltcedar are resprouting. At the first Intertribal Nursery Council meeting in 2001, the tribe asked the USDA Forest Service for help in propagating willows (Salix L. [Salicaceae]) and cottonwoods (Populus L. [Salicaceae]) to plant in these areas.

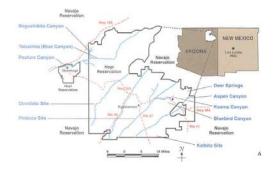




Figure 1: Riparian and wetland restoration sites on the Hopi Reservation are widely separated (A) and overrun with the exotic trees salt-cedar and Russian-olive (B). Several sites, such as Bluebird Canyon, are isolated in sandstone canyons and all the arroyo willows were observed to be the same sex (C). The exotics have been removed at some sites, such as Polacca Wash, and the area fenced to exclude cattle, but the salt-cedar is resprouting (D).

During initial visits to project areas on the reservation, we identified the principal riparian trees and shrubs: Fremont cottonwood,

Goodding's willow, coyote willow, and arroyo willow (Table 1). Tribal members also took us to remote sites where we found small stands of lanceleaf cottonwood and quaking aspen (Table 1). It is important to note that many of the wetland and riparian areas on the Hopi Reservation are geographically isolated and not always contiguous (Figure 1A). In addition, the aggressive invasion of saltcedar and Russianolive (Figure 1B) has severely reduced and separated the populations of native willows and cottonwoods. From our field observations, we suspected that several of the existing plant stands comprised only one sex, and sometimes only a single individual (Pinto and Landis 2003). One extended stand of arrovo willow along Bluebird Canyon (Figure 1C) appeared to contain only female plants, while a small grove of lanceleaf cottonwood at Deer Springs was observed to be all males (Table 1).

Removal of the salt-cedar and Russian-olive has been completed on some sites, for instance, Polacca Wash (Figure 1D). This area has also been fenced to keep cattle out and so is ready for outplanting, but the salt-cedar is already resprouting. Some test outplantings have proven successful so our challenge now is to produce enough plant materials of the proper stocktype and have them ready when the sites have been prepared.

THE PROPAGATION STRATEGY

Once the native plants had been identified, the next step was to determine where to propagate them. Because the Hopi do not have their own nursery, we contacted the Los Lunas Plant Materials Center (PMC) in New Mexico, which is operated by the USDA Natural Resources Conservation Service (NRCS) (Figure 1A). They have extensive experience growing native plants in greenhouses and outdoor fields for riparian restoration projects (Dreesen and others 2002). Most Salicaceae are vegetatively propagated with woody cuttings, and nonrooted cuttings are also widely used as live stakes or pole cuttings in riparian restoration projects. Because all members of the Salicaceae are dioecious, we had concerns about using vegetative propagation (Landis and others 2003). Using only cuttings could compromise our objective of restoring the riparian and wetland areas on the Hopi Reservation with

plants of the greatest possible genetic diversity. We wanted to produce plant communities that were self-sustaining and so decided to produce all our plant material from seeds.

Seed Production Options

Seeds of the willow family are characteristically very small and fragile and therefore store poorly (Wycoff and the Zasada 2003; Zasada and others 2003). To our knowledge, seeds of willows, cottonwoods, and aspen had never been collected on the Hopi Reservation, so there was no chance of finding a supply of local seeds. We were also ignorant of the exact flowering periods for our target species at these high elevations, which ranged from 1433 to 2073 m (4700 to 6800 ft). With limited time and funding, visiting all the projects areas to collect seeds would have been impossible.

| Table 1: List of important Salicaceae (cottonwoods, willows, and aspen) found on the Hopi Reservation, Arized | ona |
|---|-----|
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| | | | | | Sex of cut collected | tings |
|---|-------------------------|------------|-----------|-------------------|-------------------------|----------|
| Scientific name | Common name | Form | Abundance | Flowering period | Males | Females |
| Populus fremontii S. Wats. | Fremont cottonwood | Large tree | Common | Late May to June | <u>X</u> | <u>X</u> |
| <i>Populus</i> x <i>acuminata</i> Rydb. (pro sp.) [<i>angustifolia</i> x <i>deltoids</i>] | Lanceleaf cottonwood | Large tree | Very rare | Late April to May | X | |
| Populus tremuloides (Michx.) | Quaking aspen | Small tree | Very rare | Late May to June | | |
| Salix gooddingii (Ball) | Goodding's willow | Small tree | Uncommon | Late May to June | Х | |
| Salix exigua (Nutt.) | Coyote willow | Shrub | Common | May to July | Х | Х |
| Salix lasiolepsis (Benth.) | Arroyo willow | Shrub | Rare | March to April | | Х |

Collect Mature Cuttings and Produce Seeds at Nursery

So, our strategy was to identify male and female plants on the Hopi project areas during the winter dormant period, collect mature cuttings with floral buds, and root them at the Los Lunas PMC (Landis and others 2003).

We visited the project areas in late winter and early spring of 2003 and 2004 and learned to sex cottonwoods and aspen by dissecting and examining sexual buds. Because the willow buds were smaller, we made tentative sex identifications by looking for dried-up catkins. Branch ends containing floral buds (Figure 2A) were collected from trees with a pole pruner. The woody cuttings were taken to the Los Lunas PMC and rooted in containers in a greenhouse with moderate success. Coyote willow and lanceleaf cottonwood had good rooting success (80% to 90%), whereas the rooting of Goodding's willow and arroyo willow were moderately successful (75%). The sexually mature cuttings of Fremont cottonwood had much poorer rooting, about 65% after 3 months, and by the end of the first growing season, less than a third of the original cuttings were producing vigorous plants. The poor performance of Fremont cottonwood is probably because the developing flower capsules on these sexually mature cuttings created a drain on carbohydrate reserves.

Coyote willow was the most precocious and produced flowers and some seeds the first season (Figure 2B), but most cuttings only grew leaves; we had to wait until the second year to get appreciable numbers of seeds. The female arroyo willow produced some viable seeds the second year, which is interesting because we collected no male cuttings. It is possible that the seeds resulted from apomixis or hybridizing with other willows at Los Lunas PMC that flower at the same time. With only male clones of the Goodding's willows no seeds were produced. The newly stuck Fremont cottonwood cuttings produced male and female flowers but no seeds formed even after attempts to hand pollinate. In the second season after rooting, the Fremont cottonwood did not produce any flower buds or capsules. In addition, most of the mature Fremont cottonwood cuttings never developed good apical dominance and so were difficult to manage.



Figure 2: Mature Fremont cottonwood cuttings (A) rooted reasonably well and some flowered but no seeds were produced after 2 seasons. Coyote willow was the only species to produce a reasonable number of catkins and some seeds the first year (B). Our initial plan to develop willow seed production areas at Los Lunas PMC is still possible, but we had to reconsider with Freemont cottonwood.

Our initial plan was to mix the rooted cuttings from different locations, allow them to flower and cross-pollinate, and produce locally adapted but genetically diverse seeds. A detailed propagation plan is outlined in Pinto and Landis (2003). This may be possible with the willows, but the poor results with the Fremont cottonwood forced us to reconsider. In addition, concern about cross-pollination from other willows at Los Lunas PMC further reduced our confidence in developing long-term seed production areas.

Field Seed Collection

In spring 2004, we returned to the Hopi Reservation and collected seeds from some of the project areas. In particular, we wanted to increase our genetic diversity by collecting from remote locations in the western part of the reservation. We found abundant seeds of Fremont cottonwood (Figure 3A) at Pasture Canyon which, because of the great distance from Kearns Canyon and other collection areas (Figure 1A), should give us a good mix of genetics. We also collected seeds of Goodding's willow at Blue Canyon; however, because exotic willows such as *Salix babylonica* (auct. non L.) had invaded at some sites, all willows had to carefully identified before seed collection could begin (Figure 3B).

Direct seed propagation turned out to be easier than we had imagined. The seed capsules were cleaned using the procedure of the Los Lunas PMC (Dreesen 2003) and seeds were sown immediately in Ray Leach Cone-tainersTM (164 cm³ [10 in³]). Even though the Fremont cottonwood and Goodding's willow seeds were collected in mid-June, we were still able to produce large seedlings by the end of September-a growing season of only 4 months (Figure 3C). In fact, we decided to top prune these seedlings to maintain a favorable root-toshoot balance.

Dealing With Clonal Stands

As previously mentioned, we suspected that the arroyo willow and lanceleaf cottonwood stands were all of the same sex (Table 1) and perhaps even a single clone. To test this hypothesis, we collected leaf samples of arroyo willow in Bluebird Canyon and lanceleaf cottonwood at Deer Springs. Sample collection was done according to the procedure recommended by the USDA Forest Service National Forest Genetics Laboratory (NFGEL) (Hipkins 2003). When the samples were processed at NFGEL, the results confirmed our hypothesis that the extreme isolation of some of the project sites on the Hopi Reservation has resulted in clones that are genetically and sexually identical. Our ultimate objective is to locate male plants of arroyo willow and female plants of lanceleaf cottonwood and establish them in seed production plantings at Los Lunas PMC. Then, we can foster cross-pollination and produce seeds of greater diversity for our restoration efforts. Based on our experiences with Fremont cottonwood, however, we are uncertain how long it will take to produce seeds of lanceleaf cottonwood.

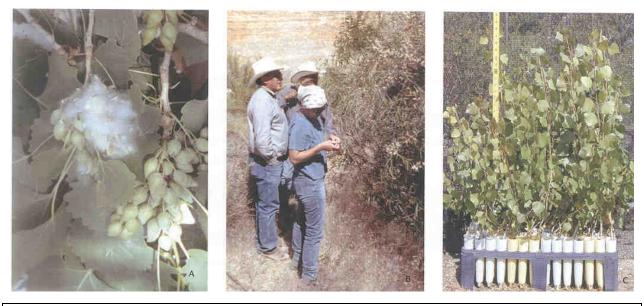


Figure 3: Collecting Fremont cottonwood seed capsules in the field proved to be relatively easy (A), but willows had to be carefully identified beforehand (B). The cottonwood and Goodding's willow seeds germinated well and produced shippable seedlings in only 4 mo (C).

THE CHALLENGE OF QUAKING ASPEN

Conventional Techniques

In the willow family, aspen is unique in that stem cuttings root poorly, so, nurseries have had better luck forcing sprouts from root sections and getting them to root (Dreesen and others 2002). We collected root sections from quaking aspen at 2 locations on the Hopi Reservation but they did not produce sprouts. This may be due to the timing of the collections or the lack of vigor in the parent trees.

Therefore, we were excited when we noticed aspen catkins on some of the trees in Aspen Canyon (Figure 4A). When they were taken to Los Lunas PMC and cleaned, however, the catkins yielded no viable seeds. This may have been due to poor pollination or moisture stress from the extended drought in the region. On a subsequent trip, some viable seeds were collected from healthier aspen stands on the surrounding Navajo Reservation. This time, the catkins did yield some viable seeds and around a dozen seedlings were grown in 262 cm^3 (16 in^3) **DeePots**TM containers and subsequently transplanted into 4-1 (one-gallon) containers for further growth.



Figure 4: The small amount of aspen seeds that we collected on the Hopi Reservation was nonviable (A), but we were able to find some on the surrounding Navajo Reservation. Using the few seedlings that came from that collection, we tried a new "stacked progpagation" technique to "bulk-up" the number of plants (B–D).

Stacked Propagation

In discussions with Larry LaFleur of Smoky Lake Nursery, we learned about a new vegetative propagation method for quaking aspen that we are calling "stacked propagation" (LaFleur 2004). This technique takes advantage of the rapid and extensive root growth of aspen seedlings and the fact that severed roots will form new shoots. We created a stack of Styroblock[®] containers with a 4-1 (1-gal) aspen seedling inserted in the top block. The lower blocks were filled with a growing medium of composted pine bark, pumice, and peat moss, and a thin layer of media was also sandwiched in between the blocks (Figure 4B). By next spring, the roots of the aspen seedling should have grown down through the cavities in the lower blocks and totally occupied all the cells. At this point, we will run a sharp knife blade between the blocks and sever the roots (Figure 4C). The pruned root systems should form new shoots,

which will develop into shippable aspen plants (Figure 4D).

In spite of its novelty, this is still vegetative propagation and so, to ensure wide genetic variation we will still try to collect more aspen seeds from the Hopi sites. We will also plant some of the Navajo aspen plants at these sites to encourage eventual cross-pollination.

DEFINING TARGET PLANT MATERIALS FOR RIPARIAN RESTORATION

Currently, we are working with the Hopi to determine the ultimate stock type for the various out-planting sites. The two primary considerations when considering target stock types for riparian restoration are hydrologic zones and the effect of erosion during flood events.

| Species | Stocktype | Container Volume | Suitable outplanting sites (See Figure 5a) | Outplanting tool |
|-------------------------|--|--|--|--------------------------|
| Fremont cottonwood | TreePots [™] PVC "tall pots" | 3875 cm ³ (one-gallon) 6145 cm ³ (375 in ³) | Far overbank and transitional zones, abandoned meanders, oxbow bends, and wide overbank | Tile spade Tile spade |
| | Poles | n/a | areas | Large-bit auger |
| Lanceleaf cottonwood | TreePots [™] PVC "tall pots" | 3875 cm ³ (one-gallon) 6145 cm ³ (375 in ³) | Far overbank and transitional zones, abandoned meanders, oxbow bends, and wide overbank areas. Upland zones for | Tile spade |
| | Poles | n/a | shelterbelts | Large-bit auger |
| Quaking aspen | TreePots™ | 3875 cm ³ (one-gallon) | Far overbank and transitional zones | Tile spade |
| | PVC "tall pots" | $6145 \text{ cm}^3 (375 \text{ in}^3)$ | | Tile spade |
| Goodding's willow | TreePots [™] PVC "tall pots" | 3875 cm ³ (one-gallon) 6145 cm ³ (375 in ³) | Far overbank and transitional zones, abandoned meanders, oxbow bends, and wide overbank | Tile spade Tile spade |
| | Pole | n/a | area | Large-bit auger |
| Coyote willow | Whips | n/a | Bank and over | Small-bit auger |
| | Ray Leach Cone-tainers TM | 1644 cm ³ (10 in ³) | Bank zone | Tile spade |
| Arroyo willow | Whips | n/a | Bank and over | Small-bit auger |
| | Ray Leach Cone-tainers™ | 1644 cm ³ (10 in ³) | Bank zone | Tile spade |

Table 2: Tentative target plant materials for riparian and wetland sties on the Hopi Reservation, Arizona.

Hydrologic Zones

For restoration purposes, the use of hydrologic zones helps to account for the presence of subsurface water at the various times of the year (Figure 5A). On Hopi lands, all plants of the willow family are found within reach of groundwater. Large trees, such as Fremont lanceleaf cottonwood. cottonwood, and Goodding's willow are located in the far overbank and transitional zones (Table 2) where their deep root systems can access water as it drops in the dry season. Quaking aspen were found in the far overbank and transitional zones where their root systems have access to the water table. The smaller shrub willows occur in the bank and overbank zones because of their extensive fibrous root systems and their flexible stems that will move with the force of the high water flows. Arroyo willow was rarer, but coyote willow was ubiquitous and the most dominant plant in the flood zone.

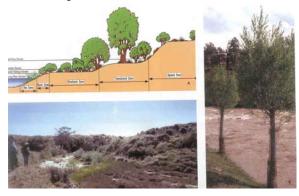


Figure 5. Hydrologic zones have been delineated for riparian areas on the Hopi Reservation (A). large stock types such as pole cuttings will be best in the flood-prone zones (B) Smaller, and less expensive, container plants would be best in wetland areas with a high water table and low erosion potential (C).

Erosive versus Non-erosive Sites

Each project site must be evaluated for the maximum effect of water erosion during high water events. This is a major factor when considering stock types because even high-quality nursery plants can be lost in a single flood event. For high water erosion sites, long pole cuttings (Figure 5B) or deep containers like PVC "tall pots" are the best option.

Tentative Stock Types

Combining hydrologic information with species characteristics, we developed a table of target plant materials for the Hopi project sites (Table 2). For cottonwoods, aspen, and the tree-type Goodding's willow, the best stock type would be the 4-1 (one-gallon) TreePot[™] or a custommade "tall pot" of 10 cm (4 in) diameter PVC pipe with a 0.6 to 0.9 m (2 to 3 ft) depth. Pole cuttings would also be an option, and we have started pole stooling blocks at Los Lunas PMC for Fremont cottonwood and plan another for Goodding's willow. For large stream courses with the highest erosion potential, pole cuttings would be outplanted in the overbank and transitional zones (Figure 5A). Because lanceleaf cottonwood and quaking aspen are relatively rare, trial outplantings of large container sizes and poles will be made in the overbank and transitional zones. Lanceleaf cottonwood may also be useful for shelterbelts around crops or structures (Table 2). Of course, the cost increases exponentially with larger stock types, and so, we may try some trial outplantings with smaller container sizes. Because of their aggressive growth habits, arroyo willow and coyote willow in the Ray Leach containers may prove useful. This may be especially effective in the wetland areas with low erosive potential, such as Polacca Wash (Figure 5C). At this site, water levels have actually increased since the salt-cedar has been removed, and they do not drop as much during the drv season.

SUMMARY: LESSONS LEARNED SO FAR

Collecting Cuttings for Seed Production

Our initial strategy of collecting mature cuttings for seed production back at the nursery is more effective for willow than for cottonwood or aspen. Many willow species are very precocious and will often produce seeds the first year or definitely by the second. Cross-pollination between species is a concern, however, especially because willows are insect-pollinated. Mature cottonwood cuttings do not root as well, and it is uncertain how long it will take to produce catkins and seeds. Still, if seed collection is difficult, this becomes a viable option and is an excellent way to maintain or even increase genetic diversity of the resultant seedlings.

Seed Collection in Field

This is the simplest and most cost-effective method if care is taken to collect from several trees over the range of project sites. With willows, careful identification is necessary to identify and exclude exotic species. Seed collection is more problematic if access to the project sites is difficult, and especially if the timing of seed production is unknown.

Propagation from Seeds

Seeds of all species in the willow family can be cleaned easily but should be sown immediately. With all species, seed propagation is the easiest and best way to maintain genetic and sexual diversity. Sowing in miniplugs with subsequent transplanting to larger containers is space efficient, but direct sowing in larger containers like Ray Leach Cone-tainers' eliminates the need for transplanting and may result in a shorter crop cycle.

Stacked Propagation This new technique is a good way to bulk-up limited plant material and should work for all species in the willow family. It still suffers, however, the major drawback of all vegetative propagation of Salicaceae -limited sexual and genetic diversity.

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Author Information

Thomas D Landis National Nursery Specialist (Retired) USDA Forest Service JH Stone Nursery 2606 Old Stage Road Central Point, OR 97502-300 nurseries@aol.com

David R Dreesen Horticulturist USDA Natural Resources Conservation Service Los Lunas Plant Materials Center 1036 Miller Street SW Los Lunas, NM 87031 david.dreesen@nm.usda.gov

Jeremy R Pinto Tribal Nursery Coordinator jpinto@fs.fed.us

R Kasten Dumroese Research Plant Physiologist kdumroese@fs.fed.us

USDA Forest Service Southern Research Station 1221 South Main Street Moscow, ID 83843