Vegetative Propagation of Key Southwestern Woody Riparian Species

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Abstract

A series of laboratory and greenhouse experiments were designed with the objective of determining effective methods of vegetatively propagating selected woody riparian species for use in restoration of Southwestern riparian habitats. Cuttings from four major southwest riparian species including Fremont Cottonwood (*Populus fremontii*), Goodding Willow (*Salix gooddingii*), Arizona Sycamore (*Platanus wrightii*), and Arizona Walnut (*Juglans major*) were collected along the Gila River in western New Mexico. Propagation studies with hardwood and root cuttings were performed. Results from these studies determined that Fremont Cottonwood and Goodding Willow could be readily propagated from dormant stem cuttings.

Nodal explants from the laboratory-grown Arizona walnut seedlings were tissue-cultured in order to develop a method to mass produce this difficult to propagate species. A nutrient and hormone solution was formulated that resulted in shoot proliferation of Arizona walnut explants *in vitro*.

Introduction

In recent years attention has shifted from phraetophyte control to the conservation of vegetation associated with water drainages in the southwestern United States. New consideration has been focused on these riparian areas as the result of construction of dams for impoundment and regulation of streamflow and from an increased public awareness for safeguarding the natural environment for wildlife habitat protection, aesthetics and recreation.

We are confronted with the human manipulation of waterflow in these riparian ecosystems to meet the ever increasing water needs of expanding human activity. Simultaneously, natural resource managers are striving to maintain or improve the habitats associated with land use activities along the natural water drainages. In many cases management of these areas includes the goal of reestablishing riparian vegetation along the streams using natural regeneration or artificial plantings to provide wildlife habitat and provide streambank stability. Since seed of key riparian plants can often be in limited supply or possess short periods of viability (Horton et al., 1960; Fenner et al., 1984; Siegel and Brock 1988), and considering the time to plant maturity, sexual regeneration is not the preferred choice of resource managers involved in restoration of riparian habitats.

Vegetative propagation is commonly used to revegetate riparian zones by using cuttings of a portion of the stem. Vegetative propagation in general utilizes a stem, root, or leaf which is placed in a favorable medium with desirable environmental conditions to initiate the development of adventitious roots. The most common approach in riparian restoration has been the use of poles cut from plants of the species of choice and the poles are placed in moist substrate, either natural or by supplemental irrigation, to provide a rooting media (Anderson et al., 1978, and York 1985). The poles are usually several cm in diameter and 2 to 3 m in length. This technique requires populations of established trees be harvested to provide materials. If large numbers of transplants are needed this technique could be potentially damaging to the parent stand of trees. In contrast, vegetative propagation using hardwood stem cuttings, which utilizes the small stems and branches, could provide large numbers of new plants for transplant material with minimal detrimental effects to parent stands. Hardwood cuttings, as defined by Hartman and Kester (1983), are those from matured, dormant stems after leaves have abscised and before new shoots emerge in the spring. Research on the effectiveness of hardwood cuttings as a propagation technique for key southwestern woody riparian species is poorly documented. The objectives of our research were to determine the efficacy of hardwood cutting techniques on woody riparian species, measure the growth responses of the regenerating cuttings and to determine the minimum amount of plant material needed to provide successful greenhouse or laboratory establishment.

Methods and Procedures

Hardwood Stem Cuttings. During early February of 1983, hardwood stem cuttings of four woody riparian species were collected from young shoots of saplings growing along a section of the Gila River in western New Mexico. Species from which cuttings were made were Fremont

Cottonwood (*Populus fremontii*), Goodding Willow (*Salix gooddingii*), Arizona Sycamore (*Platanus wrightii*), and Arizona Walnut (*Juglans major*). Cuttings from stems with extremely rank growth having abnormally long internodes, from small weakly growing interior shoots and generally stems with a large pith were avoided as recommended by Hammett (1973). Cuttings were made predominantly from lateral shoots. Our hardwood cuttings were stem tissues ≥ 5 mm in diameter and 5 to 10 cm in length. In addition to the hardwood stem cuttings, pole sections of Fremont cottonwood and Goodding Willow having stem diameters ≥ than 2.5 cm and 1 m in length were collected.

All cuttings were placed in a greenhouse where the temperature was not allowed to fall below 23 C in temperature.

Stem cuttings of dormant twigs of Fremont Cottonwood, Goodding Willow and Arizona Sycamore with terminal buds were cut into sections having 2, 4 or 6 nodes. Cuttings were placed in vermiculite filled trays so that half of the nodes were below the surface. This design involved 20 replicates of 1:1, 2:2 and 3:3 node ratios above and below the vermiculite media. Because of limited dormant stem material from Arizona Walnut, terminal stems were cut into sections having 6 nodes. Forty-eight Arizona Walnut cuttings were placed in the 3:3 configuration in the trays containing vermiculite. The vermiculite trays were kept wet by irrigation with distilled water.

The cuttings were observed daily for evidence of bud germination, but survival, and development of leaves and shoots. At the end of 12 weeks the cuttings were harvested and the number of cuttings having top growth and root development was recorded. Data were statistically analyzed between species showing regeneration using a t-test. Analysis of variance was used to test responses within a species.

Based on observations in the 1983 studies, additional dormant bud stem cuttings of Arizona sycamore and Arizona walnut were obtained from the same Gila River location and placed in the greenhouse during February of 1984. These materials were cut into 3:3 nodal sections and separated into two treatment groups. One group was soaked for 24 hours in a 50 ppm solution of the growth regulation IBA (indolbutyric acid) and the second group was soaked for 24 hours in a 500 ppm solution of IBA. These cuttings were placed in a 3:3 nodal configuration in vermiculite and kept moist. Cuttings were observed for 12 weeks and the same type of information collected in 1983 was recorded for these cuttings.

Fremont Cottonwood and Goodding Willow poles were placed in 19 liter containers filled with a standardized U.C. potting mix (Baker 1957) containing 50% sand and 50% peat moss. Five poles of each species were placed in the greenhouse. The potting mix was kept moist with distilled water irrigations. Growth data collected included the number of days for buds to germinate as well as information about shoot and root growth. After 12 weeks, the number of germinated stems, number of leaves and maximum root length (cm) was recorded.

Root Tissue Cuttings. Sprouting from root tissue provides a reproduction strategy for some riparian species. It was hypothesized that there may be a relationship between the depth of the root in the soil and its regrowth capabilities. To investigate this, root tissue was collected along the Gila River of western New Mexico in July of 1983. Root segments (10 cm in length) at depths of 0–10, 10–20, and 20–30 cm from the soil surface were cut from 2–3 year old Fremont Cottonwood and Goodding Willow saplings.

Root samples were kept moist, in dark brown polyethylene bags, during transport from the field to the greenhouse. Root segments were placed into 42 X 42 X 7 cm vermiculate-filled flats with two treatment types. One treatment consisted of complete burial of the root segments lying horizontal in the flat, while the other was placed at an angle in the flats with a portion of each root exposed to the air. There were 9 replicates of root segment per depth in each treatment for each species. The rate and quantity of shoot development were observed as was rooting activity on both treatments and species.

Tissue Culture. Arizona Walnut responded poorly to initial hardwood stem cuttings as a propagation technique. Because of this, micropropagation (tissue culture) techniques were attempted for this species. Walnut species are highly valued for their wood and, ecologically, represent an important component in some riparian areas as a food item to animals, especially squirrels (Theobald, 1983). Development of a successful tissue culture method for Arizona Walnut could provide a way to produce transplant materials of this important species.

Recent research performed by Rodriguez (1982a,b) reported success in the stimulation of shoot buds in Black Walnut (*Juglans nigra*) seeds. Based on the success of Rodriguez with *in vitro* shoot proliferation of black walnut seed explants, *in vitro* shoot proliferation of Arizona walnut stem tissue was attempted in our studies.

Axillary bud segments of approximately 1 cm in length were excised from laboratory-raised 12 week old Arizona walnut seedlings. These sections were placed in distilled water with 0.5% Tween-20 (a surfactant), surface sterilized under vacuum in a solution of 1.0% sodium hypochlorite for 15 minutes and rinsed 3 times in sterilized distilled water. Explants were then placed, under aseptic conditions, in 25 mls of tissue culture medium in 25 x 150 mm culture tubes, capped with Kaput closures.

The basic culture medium consisted of the nutrient formulation (Woody Plant Medium) developed by McCown and Lloyd (1981). Additionally, the following vitamins in mg/liter were added to the media: thiamine HCl 1.0, nicotinic acid 0.5, pyridoxine HCl 0.5, glycine 2.0, myoinositol 100, and the media also contained 2000 mg/liter of sucrose. Media pH was adjusted to 5.7 prior to adding tissue culture agar at a rate of 8.0 g/liter.

Explants were placed in this medium for 3 days and only those which were free of visible contaminants were used in additional experiments. Following this period, explants were transferred to basal medium containing various concentrations of 6-benzylamino purine (BA) and indolbutyric acid (IBA) to induce *in vitro* shoot proliferation.

The BA concentrations tested were 0, 1.0, 2.0, 4.0 and 8.0 mg/liter. Five additional treatments utilized BA at 4.0 mg/liter and IBA concentrations of: 0, 0.001, 0.01, 0.1, and 1.0 mg/liter. These concentrations of BA and IBA tested were similar to those used by Wood (1982) in studies with pecan

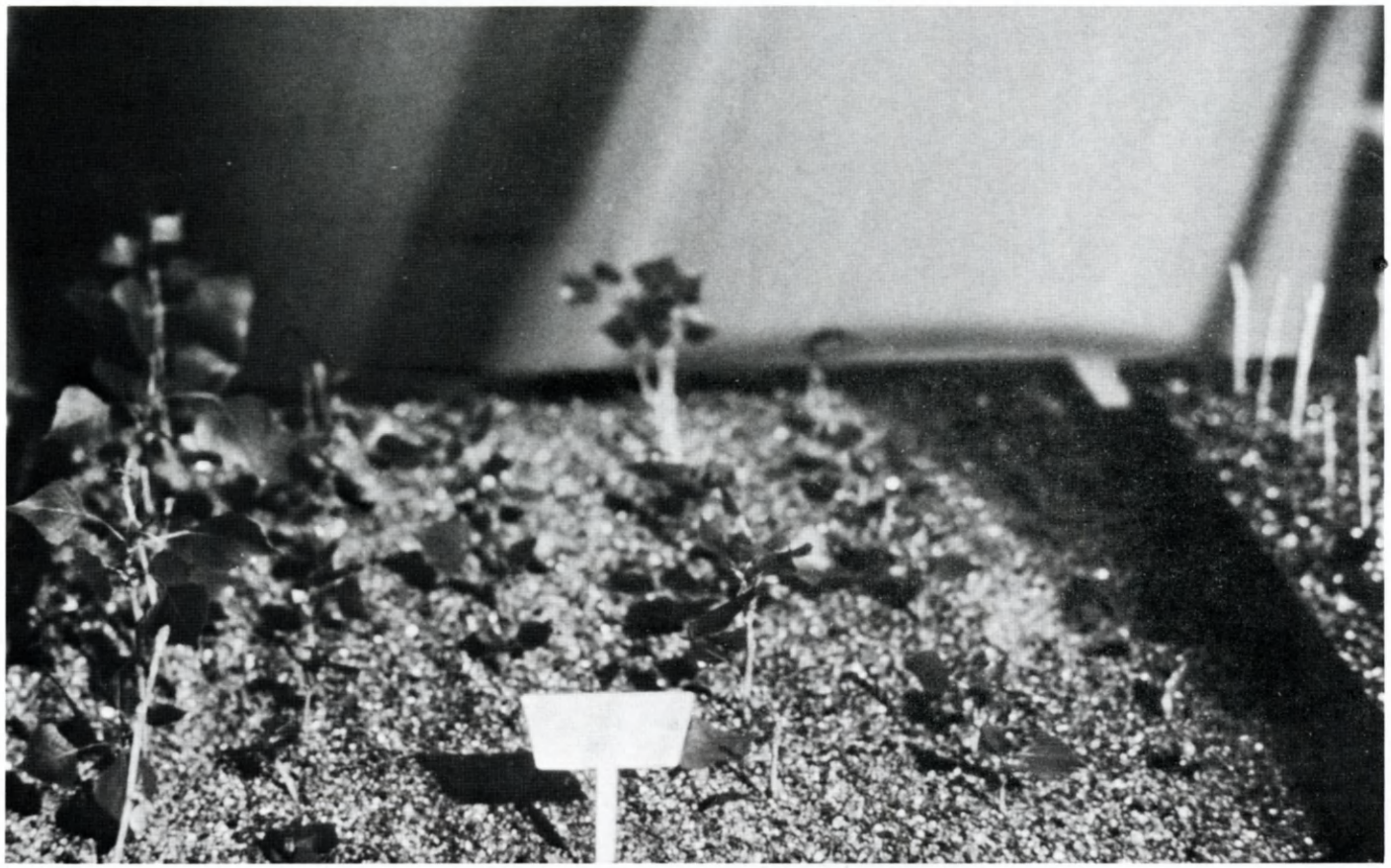

Figure 1. *Successful vegetative propagation of Fremont cottonwood from hardwood cuttings with exposed to buried bud ratios of 3:3, 2:2, and 1:1 in the photograph from left to right.*

(*Carya*). Each treatment was replicated 5 times.

Results and Discussion

Hardwood Stem Cuttings. Fremont Cottonwood and Goodding Willow were, by far, the most successful in producing growth from twig materials (Fig. 1). Fremont Cottonwood cuttings initiated bud germination within 3 days after placement in vermiculite media, while Goodding Willow began bud germination after 5 days. Arizona Sycamore, and particularly Arizona Walnut, bud germination required up to 20 days to begin, and then occurred sporadically. These latter two species proved to be difficult to propagate by the hardwood cutting method.

No statistically significant differences were found in growth responses of Fremont Cottonwood or Goodding Willow stem cuttings ($p \geq 0.05$), except for the percentage of cuttings showing growth. Twelve weeks following cutting, 95% of the Fremont Cottonwood cuttings had bud growth and survival, with each cutting having an average of 4.3, S.E. ± 0.19 leaves and a very well developed root system (Fig. 2). The majority of the Fremont cottonwood leaves were from the terminal bud. The average maximum length of roots on the Fremont Cottonwood cuttings was 13.7 S.E. ± 2.39 centimeters. Thirty-seven percent of the Goodding Willow cuttings had both root and leaf development. The average number of leaves per Goodding Willow cutting was 2.5, S.E. ± 0.14, but in comparison to Fremont Cottonwood, Goodding Willow leaves tended to develop from any node. Overall, 68% of Goodding Willow cuttings initiated root growth. The average maximum length of the Goodding Willow roots was 13.4, S.E. ± 2.83 centimeters. Arizona Walnut cuttings produced a 29% bud germination, with 19% surviving after 12 weeks. Arizona walnut did not produce any roots but did have callus tissue forming over stem cuts. Arizona Sycamore showed only 13% bud germination with no root growth. There was no significant statistical difference in growth responses comparing the number of nodes per cutting ($p \geq 0.05$).

Winter dormancy of species of the genera of Sycamore and Walnut may preclude successful results of hardwood stem cuttings as a vegetative propagation method (Hartman and Kester 1983). Attempts of hardwood stem propagation of these species before winter dormancy is broken may result only in callus tissue forming on the basal cuts. Our results tend to substantiate this. Hartman and Kester (1983) indicate that in some cases, winter dormancy restrictions can be overcome by subjecting the dormant stem tissue to cold stratification treatments, however we did not attempt this.

Arizona Walnut and Arizona Sycamore stem cuttings made in 1984 and treated with the growth regulator IBA produced no better stem growth that did untreated stems.

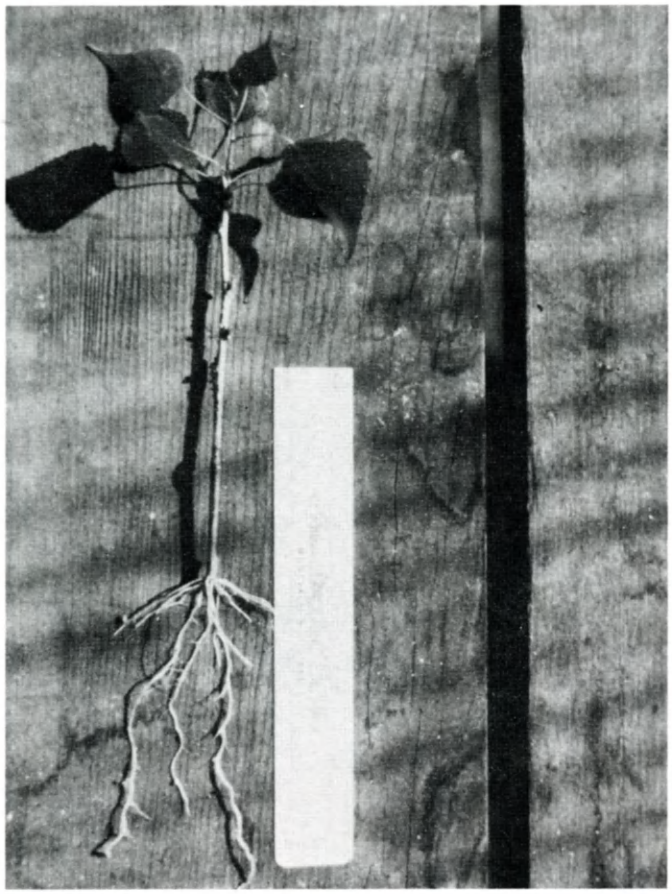

Figure 2. *Top growth and root development of a hardwood cutting of Fremont cottonwood, twelve weeks following planting.*

Figure 3. *Shoot and root growth of Fremont cottonwood from pole cuttings, twelve weeks following planting.*

After 12 weeks, 50 mg/liter of IBA improved rooting of Arizona Sycamore but not Arizona Walnut. Cuttings treated with 500 mg/liter IBA provided neither bud germination or rooting.

Pole cuttings of Fremont Cottonwood and Goodding Willow sprouted vigorously and developed extensive root systems (Fig. 3). Each species began bud break along the length of the stem within 3–5 days following planting in the soil mix and exposure to greenhouse conditions. Large diameter poles had the greater growth and development. Larger diameter poles were main stem sections from young trees while small diameter poles were from the upper parts of the main stem or branches. It is our premise that more adventitious buds are present on the lower part of the plant, which would explain our observations.

Root Tissue Cuttings. We examined the potential for root tissue as vegetative propagation material, finding that there may be a relationship between soil depth from which root cuttings were made and regrowth responses. Buried root segments of Goodding Willow were more successful in producing vegetative shoots (81%) compared to segments partially exposed to the air (62%). The greatest average shoot number per root segment of 3.3, S.E. ± 0.76, was observed for tissue taken from a 10–20 cm depth. Not only were shoots of this depth segment greater in number, they appeared to be more vigorous, having a mean height of 43.9, S.E. ± 9.4, cm 60 days following planting in the greenhouse. These root segments (10–20 cm depth) had a 31.3, S.E. ± 8.2, cm average root length. Root segments that were partially exposed to air produced fewer shoots, but the shoots present had similar growth responses in height and root formation compared to segments completely buried.

Root cuttings from Fremont Cottonwood produced very limited results. Only 2 buried segments of Fremont Cottonwood, one from a 0–10 cm depth and one from a 20–30 cm depth, produced a shoot but neither segment generated any root material by the end of the 60 day observation period.

Differences in responses of Goodding Willow and Fremont Cottonwood may have resulted from a difference in field collected tissue. Goodding Willow and Fremont Cottonwood saplings occupied a different microsite on the nursery bar area along the river's bank. The Goodding Willow was nearer the wetted perimeter (1 to 2 m) of the stream channel, while Fremont cottonwood saplings were about 3–5 m from the rivers edge. In this position on the nursery bar, the Goodding Willow was more susceptible to aggradation processes from the active channel. Compared to the Fremont Cottonwood segments, there was very active lateral rooting present on the parent Goodding Willow root segments at the time of collection. While the tissue collected from Goodding Willow was considered to be root tissue, it may have been stem tissue that had been recently buried (1 to 2 years) by deposits of fine sand and sediments. If the Goodding Willow tissue was buried stem material

with adventitious roots, it may have a greater capacity to produce shoots than would root tissues.

Tissue Culture. Arizona Walnut explants were cultured and observed for 6 weeks. After approximately 7 days in culture, all Arizona walnut explant treatments began showing signs of bud growth. Following this period responses among the various treatments began to differentiate.

All explants in the 4.0 and 8.0 mg/liter of BA showed vigorous signs of bud break and initial signs of shoot proliferation. After these primary responses, these explants ceased growth and began to dehydrate. Treatments of 0.0 and 1.0 mg/liter of BA all blackened and dried from what appeared to be a build up of phenolic compounds which may be toxic. Only treatments containing 2.0 mg/liter of BA still had active growth at the end of the 6 week observation period. At this hormone level, one explant had developed 4 shoots as well as callus growth.

Treatments which contained a base level of 4.0 mg/liter of BA plus various concentrations of IBA initially lagged behind treatments with BA only. However, for Arizona Walnut these proved to have the greatest degree of success for shoot proliferation and callus growth of Arizona Walnut. Arizona Walnut replicates with 0.001 mg/liter IBA had good bud break, but then ceased development. Sixty percent of the 0.01 mg/liter IBA-treated Arizona Walnut explants broke bud and began shoot proliferation and then discontinued growth. The 1.0 mg/liter IBA treatment of Arizona Walnut did not initiate bud growth, however, 40% of the explants developed callus that was friable and light brown in color.

The tissue culture treatment which produced the best results was at the concentration of 4.0 mg/liter of BA and 0.1 mg/liter of IBA. Eighty percent of the explants with these hormonal levels developed active shoot proliferation with as many as 6 shoots. Additionally, 50% of Arizona Walnut explants which developed shoots also had active callus growth indicating potential for root growth. At the conclusion of the 6 week study period, shoots were actively growing with no obvious lack of vigor. Treatments to stimulate root growth on tissue cultures of Arizona Walnut were not attempted in this study. However, based on our results, we are confident root development would be attainable and successful micropropagation of Arizona walnut would be possible.

Conclusions

The results of the hardwood stem tip cuttings work reinforces the idea that this method is a viable option for the production of large numbers of Goodding Willow and Fremont Cottonwood planting stock. Dormant hardwood stems are easy to acquire and economical to propagate without the need for special skill or apparatus. It would be possible to collect dormant hardwood cuttings during the winter, propagate them in the greenhouse and have plants of sufficient size by early summer to be transplanted to a revegetation area for the growing season. Further research on rooting hormones may prove advantageous in developing the same success with Arizona Sycamore that was achieved with Goodding Willow and Fremont Cottonwood.

The ease of propagation with Fremont Cottonwood and Goodding Willow poles indicates that, given an adequate water supply, this method is both easy and favorable under field conditions. Pole cutting is the most time honored method of dormant stem propagation of poplar species. Relatively fast and easy propagation of Goodding Willow from segments of the root was also found. Goodding Willow root tissue collected from an area 10–20 cm below the surface proved to be the most prolific in producing shoot and root growth.

Propagation of Arizona Walnut from dormant stem tissue with and without supplemental growth regulators did not prove to be successful. Micropropagation of Arizona Walnut through tissue culture may provide a technology for developing large numbers of genetically stable, pathogen free plants for the rehabilitation of riparian areas. The results indicate that *in vitro* shoot proliferation can be both obtained and sustained from nodal Arizona walnut explants from juvenile stem tissue. This is accomplished by supplementing the woody plant medium with a cytokinin and low concentration of auxin. In this particular research it was concluded that a base concentration level of 4.0 mg/liter of BA with 0.1 mg/liter of IBA produced the best results. Sustained shoot proliferation was not obtained either above or below these concentrations.

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