Responses of Nondormant Black Willow (Salix nigra) Cuttings to Preplanting Soaking and Soil Moisture

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Abstract

The use of willow cuttings for streambank stabilization is a common practice in riparian ecosystems throughout the United States. Many environmental factors govern the outcome of such planting. However, other factors such as preplanting treatments, planting methods, and physiological status of cuttings (dormant vs. actively growing) may also be crucial in determining the survival of willow cuttings. Actively growing (nondormant) Black willow (Salix nigra) cuttings, 30 cm in length and 1 cm in diameter at the base, were subjected to three soaking treatments (0, 7, and 15 days) prior to planting. Following the initial treatment, cuttings were grown in a greenhouse in pots under three soil moisture regimes (well-watered but not flooded, permanently flooded, and intermittently flooded). Plant gas exchange, growth, biomass, and survival were measured. Results demonstrated that soaking for 7 days was beneficial to early development of cuttings in the well-watered (control) soil moisture regime, enhancing percent bud flush and survival significantly. However, 15 days of soaking proved to be detrimental to survival of cuttings irrespective of soil moisture regimes. Results also demonstrated that the beneficial effects of 7-day soaking were limited to the well-watered soil moisture regime but not to the flooded or intermittently flooded regimes. Soaking nondormant cuttings may be worthwhile if the planting site is likely to present ample soil moisture but nonflooded conditions to the transplanted cuttings.

Key words: erosion control, riparian restoration, Salix nigra, soaking, soil moisture, streambank stabilization.

Introduction

The use of willow (Salix spp.) cuttings for streambank stabilization and rehabilitation is a common practice in many riparian ecosystems throughout the United States. The use of plant materials, such as willow cuttings, provides a beneficial alternative or addition to typical bank stabilization practices incorporating solely “hard” materials such as riprap or similar materials. Previous studies have indicated that cutting survival rates of 29–34% are needed for successful bank stabilization (Watson et al. 1995). For streambanks planted at a standard density of 0.9-m spacing, a survival rate of about 50% after 2 years is considered to provide adequate stabilization (Shields et al. 1995). However, survival rates as low as 10% have been reported for some willow species (Wolfe 1992).

Field studies and laboratory experiments have identified factors critical for willow survival such as drought, low soil redox potential (Eh), and soil texture (Pezeshki et al. 1998; Schaff et al. 2003). However, the physiological status of cuttings prior to planting (actively growing vs. dormant), preplanting treatments such as soaking in water, and planting methods may also be crucial in determining the success of willow cuttings.

Black willow (Salix nigra Marshall.) is a flood-tolerant species found within the floodplains of the southeastern United States. It produces abundant adventitious roots (Krasny et al. 1988) and is thereby capable of rapid establishment in habitats where soil moisture is abundant. Production and extension of a substantial root mass has been reported as a critical attribute that allows cuttings to avoid water stress (Pallardy & Kozlowski 1979). Furthermore, abundant water in the propagation medium may enhance water absorption by the unrooted cuttings thereby helping to restore turgor (Grange & Loach 1983). It appears that a preplanting treatment that enhances tissue water content may promote root initiation, development, and early survival of willow cuttings if such cuttings were dormant at harvest.

Previous reports on cuttings from some woody species, including dormant Black willow cuttings, indicated that soaking in water prior to planting may significantly enhance shoot and root growth (Bloomberg 1963; Petersen & Phipps 1976; Phipps et al. 1983; Schaff et al. 2002). For most woody species, these positive responses have been attributed to the improved water content of the cuttings as well as root and shoot initiation during the soaking period. Furthermore, these benefits to cuttings may depend on the length of soaking prior to planting as well as the soil moisture regime.
moisture regime encountered during the period immediately after the transplanting.

For dormant Black willow cuttings, soaking for 10 days prior to planting enhanced root and shoot production, whereas 3 days of soaking had no detectable effects (Schaff et al. 2002). However, it is not known whether soaking periods longer than 10 days would be beneficial. In addition, although the benefit of soaking dormant cuttings is apparent, it is not known if similar responses will occur in actively growing (nondormant) cuttings. Clearly, the physiological status of nondormant cuttings is different from that of dormant cuttings (Lang et al. 1985; Lang 1987). From the management perspective, the use of actively growing cuttings, if successful, would expand the time frame for restoration activities that are now limited in many regions, including the southeastern United States, to a relatively short period in the winter when plants are dormant. Further, in many regions, the period of dormancy coincides with seasons when high flows occur, raising the probability that recently planted cuttings will be washed away before they are secured by roots and shielded by leaves and shoots. Use of actively growing materials, especially when coupled with irrigation during the period of establishment, would allow establishment of plant materials during warm, low-flow periods prior to being tested by high, erosive flows.

Based on the above discussion, a greenhouse study was conducted to explore the effects of preplanting soaking duration on survival and growth of actively growing (nondormant) willow cuttings. After soaking treatment, cuttings were planted across a range of soil moisture regimes. The specific objectives included quantifying the effects of various soaking durations on root initiation, root elongation, shoot production, and survival of nondormant Black willow cuttings.

### Materials and Methods

Cuttings from actively growing trees were collected from a localized, naturally occurring Black willow population located at the University of Memphis Loosahatchie Biological Station in Bartlett, Tennessee, U.S.A. The cuttings were taken manually and measured 30 cm in length and 1 cm in diameter. The cuttings were randomly assigned to three soaking treatments: control (nonsoaked), soaked for 7 days, and soaked for 15 days. The 7- and 15-day-soaking cuttings were placed in plastic tubs filled with tap water and soaked for the specified time. The nonsoaked cuttings were planted immediately after harvesting. Aeration pumps were used to maintain stable dissolved oxygen levels within the tubs during the soaking periods. The cuttings, including the nonsoaked group, were planted in 2.5-L pots that were filled with soil collected from the A horizon of a Falaya Series silt loam from an abandoned agricultural field in Shelby County, Tennessee, U.S.A.

Pots containing the cuttings were placed in a ventilated, air-conditioned greenhouse. Three soil moisture regimes were initiated consisting of control (well-watered but not flooded, C), intermittent flooding (I), and continuous flooding (F). Pots assigned to treatment C were watered daily to field capacity with 300 ml of tap water. Pots assigned to treatment I were flooded to 5 cm above soil surface for 4 days and drained for 10 days to mimic natural hydrologic conditions. Pots assigned to treatment F were flooded permanently with water levels maintained at 5 cm above the soil surface.

The experimental design consisted of a 3 × 3 factorial, complete randomized block design in order to compensate for any variation in environmental conditions within the greenhouse. There were a total of 378 cuttings, with 42 randomly selected cuttings per treatment. Subsamples of seven randomly selected cuttings were harvested on days 0, 4, 10, 17, 42, and 48 for biomass measurement. Cuttings were washed and separated into foliage and root. The numbers of buds, root primordia, and roots were recorded along with the fresh and dry weights (g) of individual biomass components. Prior to planting, no roots or root primordia were present on soaked or unsoaked cuttings. Fresh weights were determined immediately after harvesting. Samples were then oven-dried at 80°C to a constant weight in order to determine dry weights. Leaf area was measured using a Leaf Area Analysis System (Decagon, Inc., Pullman, WA, U.S.A.).

Plant gas exchange measurements, including net photosynthesis (Pn), and stomatal conductance (gs), were conducted on intact leaves from the upper portion of branches of six randomly selected cuttings per treatment using a portable field photosynthetic system (CIRAS1, PP Systems, U.K.). These measurements were conducted between 0800 and 1100 hr on days 28, 42, and 48.

Soil measurements included soil Eh. Eh was measured using platinum-tipped electrodes placed in the pots at 10–15 cm below soil surface in five randomly selected pots per treatment. A portable pH/mV meter (Orion Model 250A, Orion Research, Inc., Boston, MA, U.S.A.) and a calomel reference electrode were used to determine soil Eh (Patrick & DeLaune 1977; Pezeshki & DeLaune 1998).

Final analysis of the data employed general linear models and Tukey’s studentized range test procedures of the SAS system (SAS Institute, Inc., Cary, NC, U.S.A.) to determine any significant differences across treatment groups under various soaking duration and soil moisture regimes. Survival data were analyzed using nonparametric procedures of the SAS system. Repeated measures analyses (SAS 1990) were used in order to test differences in means for all gas exchange measurements.

### Results

Cuttings soaked for 15 days produced significantly lower amounts of shoot and root biomass and numbers of buds and roots, and none of them survived longer than 42 days. Cuttings soaked for 7 days produced significantly higher...
numbers of buds and roots and displayed a higher rate of survival than unsoaked cuttings (Table 1).

There was a close relationship between soaking and the percentage of cuttings that flushed buds. Depending on the soaking treatment, between 7.2 and 92.9% of the cuttings flushed (Table 1). The highest bud flush was noted in I0 and I7 treatments, whereas the lowest flushes occurred in the 15-day-soaking treatments (C15, I15, F15). The control group (C0) flushed 35.7% (Table 1).

Clearly, soil moisture regimes led to substantial differences in soil Eh conditions in various treatments. In controls, mean Eh was +470 ± 25 mV. Thus, soils in this treatment remained aerated with Eh greater than +350 mV. In intermittently flooded pots, mean Eh varied depending on whether pots were drained or flooded. For example, mean Eh was +290 ± 50 mV during the flooded periods representing reducing conditions, whereas it was +410 ± 25 mV during the drained periods. For each draining period, soil Eh recovered to aerated condition shortly after draining. In pots subjected to treatment F, mean Eh was +170 ± 25 mV. The latter treatment presented the most reducing condition for plants. Creating a permanently reducing condition for plants.

Survival data for treatments I and F followed a similar pattern with the 7-day-soaked cuttings and the unsoaked cuttings, both notably outperforming the 15-day-soaked group. Again, 15-day-soaked cuttings did not survive past day 42. Survival rates varied (χ^2 = 48.22, p < 0.001) across treatment combinations (Table 1). In the control, 7-day-soaked cuttings had the highest survival rate (85.7%) after 48 days, followed by the 0-day-soaked cuttings (57.1%), whereas the survival was lowest (0%) for the 15-day-soaked cuttings. Again, 15-day-soaked cuttings did not survive past day 42. Survival data for treatments I and F followed a similar pattern with the 7-day-soaked cuttings and the unsoaked cuttings, both notably outperforming the 15-day-soaked group.

No significant differences were found for either Pn rates or gw in response to soaking or soil moisture regimes using multivariate analysis of variance (MANOVA), repeated measures procedures (Wilk's lambda = 0.9846, p = 0.8650). However, there was a significant interaction between flooding and soaking on leaf area (F = 7.76, p = 0.0006). Cuttings under treatments I7 and C7 had greater leaf areas than those under treatments F7 and F0. There were no significant differences between treatments C0 and I0 and the other treatments (Fig. 2). However, higher shoot dry weight (Fig. 1) and leaf area (Fig. 2) in the unsoaked and 7-day-soaked groups indicated a greater capacity for carbon fixation at the whole plant level compared to the 15-day-soaked plants.

The dry weights produced for various biomass components were consistently greater in the 0- and 7-day- than in the 15-day-soaking treatment. Cuttings soaked for 15 days produced fewer root primordia and roots than cuttings soaked for 0 or 7 days (p < 0.0001), representing a substantial decrease in root surface area for water and nutrient absorption.
Discussion

For actively growing (nondormant) Black willow cuttings, 15 days of preplanting soaking proved to be harmful irrespective of the soil moisture regime during the posttransplanting period. Soaking cuttings for 15 days followed by transplanting them into flooded or drained soil moisture regimes led to reductions in all measured biomass components, growth, and survival. In contrast, soaking for 7 days enhanced performance as measured by certain parameters such as number of buds produced. However, the beneficial effects were limited to the control soil moisture treatment. For instance, when soil moisture was maintained at field capacity, percent bud flush and number of buds were higher for the 7-day soaking regime as compared to control by factors of 5 and 2.3, respectively. Hansen and Phipps (1983) noted that shortly after transplanting, nearly 100% of cottonwood cuttings soaked for 10 days had flushed, whereas no flushing occurred for cuttings that had not been soaked. Similarly, soaked cuttings consistently flushed earlier and produced longer shoots when grown in soil water potential ranging from field capacity to moderately drained in comparison to nonsoaked cuttings. Schaff et al. (2002) found no soaking effects on the percentage of dormant Black willow cuttings that flushed buds or the average number of buds produced. Their finding was in contrast to the current study that demonstrated significant effects of 7-day soaking on percent bud flush and number of buds in nondormant cuttings under treatment C. The difference is attributed to the physiological status of the cuttings used (i.e., the previous study used dormant cuttings, whereas the present study utilized actively growing cuttings).

Previous studies also reported increases in the number and dry weight of roots for Populus spp. when cuttings were subjected to a soaking treatment (Petersen & Phipps 1976; Phipps et al. 1983). Schaff et al. (2002) reported that dormant Black willow cuttings soaked for 10 days...
produced more roots and more root biomass than did cuttings that were not soaked or soaked for 3 days. Again, their findings contrast with the present study that found no benefit from soaking on root dry weight in the 7-day-soaked group and significant decreases in root production in the 15-day-soaked group. The present results highlighted the differences in response of cuttings based on their initial physiological status.

The differences in shoot and root responses to soaking noted between the dormant cuttings used in previous studies and the actively growing materials utilized in the present study are interesting. These differences highlighted the importance of physiological status of Black willow cuttings in governing subsequent root and shoot development. Differences in responses noted are likely due to a number of factors including the variation in growth regulator concentrations and/or ratios and the availability of carbohydrates. Roots initiated on cuttings, by definition, are known as "adventitious roots." Adventitious rooting phenomenon has been primarily attributed to auxin, a root-inducing hormone, although other plant hormones such as ethylene have been implicated as well (Haissig & Davis 1994). Adventitious root initiation is governed, in addition to the genetic control, by a balance of internal substances such as hormones, carbohydrates, and nitrogenous compounds and many cofactors (Nada et al. 1971; Friend et al. 1994; Haissig & Davis 1994; Houle & Babeux 1998). Furthermore, it has been reported that rooting capability of cuttings may be governed by a critical balance between the nutritional factors as well as hormones; thus, roots may not develop if there is an imbalance of these factors (Nada et al. 1971). A dormant cutting is

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 10</th>
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<tbody>
<tr>
<td><strong>F Value</strong></td>
<td><strong>p Value</strong></td>
<td><strong>F Value</strong></td>
</tr>
<tr>
<td>Shoot DW</td>
<td>3.12 (I)</td>
<td>3.34 (I)</td>
</tr>
<tr>
<td>Root DW</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Number of buds</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>Number of root primordia</td>
<td>4.59 (F)</td>
<td>5.96 (S)</td>
</tr>
<tr>
<td>Number of root primordia</td>
<td>8.66 (F)</td>
<td>5.96 (S)</td>
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<tr>
<td>Root-to-shoot ratio</td>
<td>n.s.</td>
<td>n.s.</td>
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Letters in parentheses indicate source of statistical significance at $p < 0.05$ (F = flooding effect; S = soaking effect; and I = interactive effect between flooding and soaking).

Figure 2. Leaf area (cm$^2$ per plant) for cuttings of Black willow (Salix nigra) subjected to various soaking duration and soil flooding regimes at the conclusion of the experiment (day 48). Soaking duration consisted of no prior soaking (0 days) and 7 days of soaking. Soil flooding regimes were: well-watered but not flooded (control, C), flooded (F), and intermittently flooded (I). Data for the 15-day soaking treatment are not presented due to the absence of leaves. Different letters represent significant differences across all treatments ($p < 0.05$).
likely to have a greater pool of carbohydrates than an actively growing cutting.

In the field, enhanced adventitious root initiation and elongation provides the opportunity for the transplanted willow cuttings to exploit and expand into various soil horizons. However, clearly such a response would be dependent on the carbohydrate-partitioning patterns for the cutting at the early phase of root and shoot development. There is a general lack of information on the pattern of root development on cuttings and its relationship to carbohydrate partitioning. It is assumed that carbohydrate availability is important in initial root development, but continuation of root growth may be most dependent on current photosynthate supply (Friend et al. 1994). Following root initiation, maintenance of a balance between root and shoot is critical to the survival of plant propagules.

The soil moisture condition of the media in which cuttings are transplanted may also be an important factor as was noted in the present study. Previous studies (Schaff et al. 2002) reported that Black willow cuttings soaked for 10 days and grown across a wide range of soil moisture conditions had improved growth, biomass production, and survival. However, in the present study it was obvious that significant effects of 7-day soaking on percent bud flush and number of buds were detectable only if the medium presented a well-watered but drained soil moisture regime (treatment C). Again, there was a profound difference in responses between dormant cuttings of Black willow as reported in the literature (Schaff et al. 2002) and the actively growing cuttings used in the present study.

Typically, restoration projects plant willow cuttings in the spring but collect the materials while plants are dormant. Thus, soaking-induced increases in root initiation and growth noted for dormant materials coupled with favorable soil moisture conditions may enhance root extension into aerated but moist soils as reported earlier (Schaff et al. 2002). Such root expansion creates additional opportunity for water and nutrient uptake and growth, leading to improved flood and drought avoidance capabilities later in the growing season. However, if cuttings were collected later in the spring when the plants are not dormant, the response may be quite different as was noted in the present study. Houle and Babeux (1998) reported that when cuttings of Salix planifolia were collected from actively growing trees, rooting ability was low compared to cuttings collected when trees were dormant. The authors attributed this finding to the variations in the level of stored carbohydrates. However, as mentioned above, the relationship between carbohydrate levels and formation of adventitious roots remains to be documented (Phipps & Netzer 1981).

Soaking treatment, depending on the dormancy status of a cutting, may facilitate root growth of cuttings. For instance, placing woody cuttings in water led to rapid root development for some species (McNight & Biesterfeldt 1968; Hansen & Phipps 1983; Phipps et al. 1983); consequently, root primordia developed to a stage where functional roots emerged shortly after transplanting. In addition, a successful adventitious root establishment requires establishment of a vascular link between the carbohydrate source and the newly developed root that is also related to the anatomical characteristics of the cutting (Vieitez et al. 1980; Friend et al. 1994). Thus, formation of new roots close to the existing vascular tissue is an important factor. For instance, preformed root initials occur in leaf gaps of nodes close to vascular tissue in Salix spp., an advantageous position allowing rapid vascular connections for the newly formed root (Friend et al. 1994). This characteristic should be the same for the dormant and nondormant Black willow cuttings. Early emergence and rapid root elongation have been shown to result in significant increases in growth and survival in cuttings of some woody species (Tschaplinski & Blake 1989; Flygh et al. 1993). Rein et al. (1991) reported that the presence of as few as one or two roots substantially improved midday xylem pressure potential compared to unrooted cuttings. Soaking increases the total water content of the cutting, allowing water stress avoidance prior to and during early stages of establishment (Pallardy & Kozlowski 1979; Grange & Loach 1983; Hansen & Phipps 1983; Ikeda & Suzaki 1986; Deka et al. 1988). Cuttings of Populus spp. soaked for 10 days prior to transplanting in a range of soil moisture produced roots irrespective of soil moisture regime (Hansen & Phipps 1983). In contrast, soaking for 4 days enhanced rooting when cuttings were grown in moist soils but not in dry soils indicating improved water content of the cuttings that resulted from an extended soaking period.

Conclusions

Soaking for 15 days proved to be detrimental to the survival of cuttings irrespective of the posttransplanting soil moisture regime. However, soaking for 7 days appeared to be beneficial as measured by certain parameters. The benefits of 7-day soaking were dependent on the soil moisture conditions encountered by cuttings. Therefore, soaking of nondormant Black willow cuttings selected for planting should be considered only if the planting site is likely to present initial conditions of ample but nonflooded soil moisture. Because riverbanks have a moderate to steep slope, such moisture conditions are more likely to be found at midbank rather than at the top or toe of the bank.

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LITERATURE CITED


