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Vegetative propagation in the emergent macrophyte American water willow (*Justicia americana* L. Vahl)

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INTRODUCTION

Over the past few decades, numerous attempts have been made to establish aquatic vegetation within littoral zones of freshwater lakes and reservoirs as a means of enhancing the productivity of recreationally important fishes, improving water quality, and/or minimizing shoreline erosion (e.g., Qui et al. 2001, Strakosh et al. 2005). The success of these plantings is often dependent on grazing intensities from herbivores that remove important aboveground structures or uproot vegetation through benthic feeding, and the ability of plants to survive dynamic hydrologies (Nishihiro et al. 2004, Strakosh et al. 2005, Touchette et al. 2007). One prominent North American plant that seems to be somewhat tolerant of these biotic and abiotic perturbations is American water willow, *Justicia americana* L. (Vahl.) (Lewis 1980, Hill 1981, Strakosh et al. 2005, Touchette and Frank 2009). This tolerance is likely fostered by its complex network of belowground structures and its remarkable ability to regenerate new aboveground tissues following major disturbances (Fritz et al. 2004a).

The native range of water willow includes the eastern half of North America from Quebec to Florida and westward into Texas (Niering and Olmsted 1979). In some parts of the Midwestern United States, water willow is either threatened (Michigan) or endangered (Iowa) and is classified as a species of concern in Canada. In North Carolina, it is considered a principle habitat species for the endangered Cape Fear shiner (*Notropis mekistocholas*; Hewitt et al. 2009), and although restoration programs involving water willow along the middle Cape Fear River have yet to be initiated, it is likely to be a necessary component in maintaining viable Cape Fear shiner populations in the future.

In shallow lake and river systems (<1.5 m), water willow is among the first colonizers of barren substratum where it often stabilizes the shoreline with its massive network of belowground structures (Lewis 1980, Strakosh et al. 2005). This colonial plant quickly spreads along shorelines, often forming highly dense monocultures (Lewis 1980, Hill 1981). Because of its aggressive rhizomatous growth, in some regions water willow is considered a pest species (Strakosh et al. 2005). Nevertheless, this concern is offset by the advantages water willow provides in terms of enhancing streambed and shoreline stability and modifying habitat to support associated organisms (Keiper et al. 1998, Fritz et al. 2004b). In hydrologically dynamic systems, water willow has been shown to tolerate scouring floods, intense wave action, and fluctuating water levels (Penfound 1940, Lewis 1980, Fritz and Feminella 2003, Strakosh et al. 2005). Therefore, in barren littoral areas, with comparatively intense hydrologies (e.g., high water velocities or varying water levels), water willow may be a desirable candidate for establishing emergent vegetation. The success of water willow in these areas, however, may be dependent on its size, health, and maturity at the time of planting. In regions where natural populations of water willow are limited and/or protected, greenhouse propagation could allow for the mass production of a large number of planting units from only a few cuttings.

The purpose of this study was to experimentally evaluate culture techniques for rapid greenhouse propagation of water willow from stem cuttings. More specifically, we compared how flooded and water-saturated conditions influenced new shoot production and growth, and evaluated the use of a root-promoting hormone for enhancing root growth.

MATERIALS AND METHODS

In early July 2009, approximately 150 cuttings were collected from a midmarsh region of Badin Lake (a reservoir near Albemarle, NC). Plants were cleaned and processed by removing chlorotic or necrotic leaf tissue, and stems were cut to length (approximately 45 cm). The initial numbers of leaves and nodes per cutting were 12 to 22 and 7 to 9, respectively. Six water willow cuttings were equally selected from harvested stock and placed in each tray (standard 1020 flats) containing 2 L of sand. A total of 20 trays were placed in a randomized complete block design within a glass research greenhouse, with 4 different treatments composed of (a) wet sand: moist well-drained sand (a widely used propagation substrate for aquatic plants; Baca and Ballou 1989, Schaff et al. 2003); (b) flooded sand: water levels maintained at 4 to 5 cm above the sand substrate, (c) wet sand + hormone: well-drained sand with root-promoting hormone; and (d) flooded sand + hormone: flooded trays with sand and root-promoting hormone (n = 5 trays for each treatment). The root-promoting hormone was a readily available product (Rooting Hormone, Green Light Co., San Antonio, TX) common

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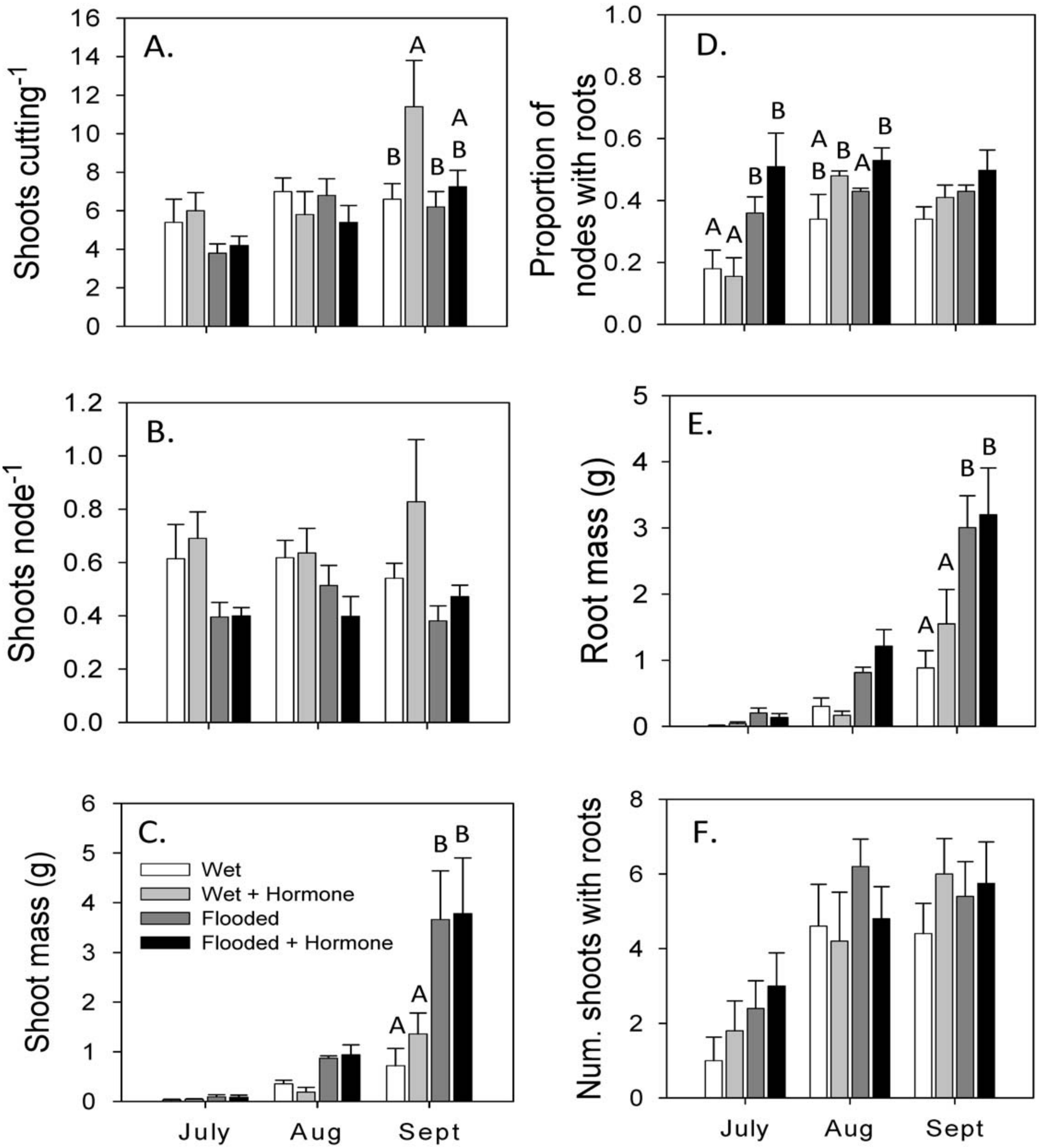

Figure 1. American water willow shoot and root characteristics including (A) new shoots per cutting, (B) new shoots per node, (C) total new shoot mass, (D) number of nodes with roots, (E) total root mass, and (F) number of shoots with roots. Data include wet sand without hormone (white bars), wet sand with hormone (gray bars), flooded sand without hormone (dark gray bars), and flooded sand with hormone (black bars). Data are presented as means ± 1 SE. Means with different letters are significantly different according to Tukey-Kramer post-hoc analyses ($\alpha = 0.05$).

in many horticultural supply stores, and contained 0.1% of the active phytohormone indole-3-butyric acid (a synthetic auxin similar in structure to indole-3-acetic acid). The hormone was applied to each node according to the manufacturer's instructions. To prevent nutrient growth limitation, 4 g of a slow release fertilizer (Osmocote 14-14-14) was added to each tray at the time of planting. Throughout the study, trays with cuttings were watered for 10 min, 3 times a day (2:00 AM, 10:00 AM, and 2:00 PM) with overhead misters. Individual watering delivered approximately 0.5 cm of water to each tray, totaling 1.5 cm of water d^{-1}. At this rate, wet-sand treatments remained moist throughout the study.

At monthly intervals, a single cutting was removed from each tray and measured for new shoot production; measurements included number of shoots per cutting, number of shoots per node, and total new shoot mass. We also considered root development by measuring total root mass, number of nodes with developing roots, and number of new shoots with roots. Mass of plant tissue was determined by oven drying at 60 C until constant weight.

All growth parameters were evaluated for equal variance and normality (Kolmogorov-Smirnov test). Shoot and root weights were log transformed, and the number of shoots per cutting was square-root transformed to satisfy normality prior to statistical comparisons. Data were analyzed using a 3-way repeated measures ANOVA (GLM; SAS 9.1) with measured shoot and root parameters as dependent variables, and hormone, water conditions, and time as independent variables. A Tukey-Kramer post-hoc analysis was performed when significant differences were reported ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Regardless of treatment, water willow cuttings were capable of producing new vegetative shoots. After 3 months, the mean number of new shoots per cutting was between 6.2 and 11.4; plants in the wet sand + hormone treatment had the highest numbers (Figure 1a). Most of the shoots emerged during the first month, and subsequent increases in plant mass were primarily due to increases in shoot size and/or continued apical growth of the original cutting (data not presented). There was a significant shoot response associated with water treatments, wherein flooded treatments had less shoot proliferation by the end of the study ($p = 0.030$; Figure 1a). While statistically significant, these outcomes are mostly attributed to the markedly higher performance observed in the wet + hormone treatment rather than the overall performance of both wet-sand treatments collectively (Figure 1a). Nevertheless, this response is consistent with field observations, where new shoot production was reduced by 28% when growing in 2 cm of water in comparison to plants lying directly on the substratum (Lewis 1980). Shoot mass was significantly greater in flooded treatments than in wet-sand treatments at the end of the study ($p < 0.0001$; Figure 1c). Shoots were more than 3 times larger in flooded sand (3.72 ± 0.65 [SE] g of new shoot per cutting) than in wet sand (1.04 ± 0.28 g). This suggests that growth in flooded conditions may enhance overall productivity, resulting in larger and presumably healthier plants.

Unlike results for shoots, the number of nodes that developed roots was affected by both flooding and root-promoting hormone. In this case, there was a slight, albeit significant, positive response with hormone applications ($p = 0.022$; Figure 1d) which was most pronounced in the second month (Aug). This difference, however, was no longer evident by September. Greater root mass was observed in flooded plants (3.10 ± 0.36 g of new root tissue per cutting) than plants in wet sand (1.21 ± 0.29) at the end of the study ($p < 0.0001$; Figure 1e). There were no significant differences in the number of new shoots with roots between any treatments. Root development seemed to lag behind shoot development, and the onset of root growth was likely contingent on the initiation of shoots.

Based on the results of this study, we conclude that water willow is readily propagated from stem cuttings, which is consistent with observations made in natural settings (Lewis 1980). Most cuttings produced new shoots, including those that developed mild necrosis in leaf and stem tissue. Shoot and root mass was significantly higher for plants cultured in flooded sand than for plants in wet sand. While we observed a significant response in shoot and root development following some hormone applications, we believe that these benefits were not substantial and could not justify the added expense and time necessary to apply root-promoting hormone to water willow nodes.

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