Development of a Black Willow Improvement Program for Biomass Production in the Lower Mississippi River Alluvial Valley

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Abstract
Black willow (*Salix nigra* Marsh.) has the potential to be a significant feedstock source for bioenergy and biofuels production in the Lower Mississippi Alluvial Valley (LMAV). This potential is based on a number of primary factors including rapid growth, ease of vegetative propagation, excellent rooting, and the ability to regenerate from coppice following harvest. To date, there has been no directed black willow improvement effort for the LMAV and production rates of this species in dedicated energy plantations is unknown. The focus of this program is to identify genetically superior black willow clones and define planting stock for use in regeneration of marginal agricultural sites. The Mississippi State University Forestry Department and the USDA Forest Service Center for Bottomland Hardwoods Research formed a joint venture in 2008 to pursue this effort. The initial selection strategy incorporated five geographic areas, four stands within each area, and five clones within each stand. The five geographic areas included two along the Mississippi River and one each along the Atchafalaya, Trinity, and the Brazos Rivers. From each stand 5-8 one to two year-old stems were collected during the winter of 2009. Over a two-year period a total of four screening trials were established. Data from ages one and two have provided insight into geographic origin performance and heritability. These early results allowed us to design a more highly replicated clone test of the better performing clones as well as to increase selections for the base population.

Keywords: Black Willow, Selection Strategy, Geographic Origin, Clone Testing

Introduction
Various willow species and their hybrids (*Salix spp*.) have been used in Europe and proposed for use in the northeastern United States as a fast growth forest species for the bioenergy and biofuels production (Abrahamson 1998; Dickmann 2006; Kopp et al. 2001). Although black willow (*Salix nigra* L.) grows throughout the southern United States, it has received very little attention as a biomass species. In this area and especially in the Mississippi Alluvial Valley (MAV), the majority of the research has focused on eastern cottonwood (*Populus deltoides* Bartram ex Marsh.), which was used by the pulp and paper industry. While various *Populus* species and their hybrids are still being evaluated for biomass production, the sites now being examined for large-scale production are considered "marginal". The reasoning behind the use of marginal sites for biomass production is to avoid further controversy in the "food versus fuel" debate; thus productive agricultural sites are simply avoided. Marginal sites include those that are characterized by heavy clay soils and prolonged flooding. Although, eastern cottonwood may survive on these types of sites it will not reach its biological potential. While black willow is adapted to these types of sites biomass production of plantation grown black willow is currently unknown. But with the development of silvicultural tools and the inclusion of genetically improved planting stock, black willow may prove to be a viable biomass species on these sites.

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Black willow is easily vegetatively reproduced from stem cuttings allowing the selection of superior genotypes and exploitation of genotype by environmental interactions. The exceptional rooting characteristics of black willow dormant unrooted cuttings typically result in extremely high survival rates. Benefits from high survival rates could include reduced competition control, uniform production rates, increased yields during the first rotation, and greater potential from coppice regeneration (Ceulemans et al. 1996; Kuzovkina and Quigley 2005).

The main objective of the program was to define genetically superior genotypes for use in biomass plantations along the lower Mississippi River Alluvial Valley.

Methods

The initial sampling scheme for the program included five geographic areas (i.e. sources), all of which were found along four southern rivers (i.e. Mississippi, Atchafalaya, Trinity, and Brazos Rivers). Two areas were sampled along the Mississippi River, with one located near Rosedale, MS and the other near Tunica, Mississippi. The area sampled on the Atchafalaya River was located near Patterson, LA and was the most southern location of the five areas. The two locations along the east Texas Rivers of the Trinity and the Brazos were near Antioch, TX and College Station, TX, respectively. From each of the five geographic areas four stands were randomly selected and from each stand at least five randomly selected clones were collected. An additional 13 clones were also randomly collected, resulting in 113 total clones. During the sampling, a number of sandbar willow clones were mistakenly collected and later isolated in the stoolbed and the tests. All of the clonal material was collected during the winter of 2008-2009 from either one or two-year-old juvenile willows, and placed into a stoolbed during the spring of 2009. During January 2010, whips from each clone were harvested and used either in establishment of two clonal screening trials in 2010 or in the formation of a new stoolbed. At this time the sandbar willow collection was isolated to ensure a separation of the two species. With two stoolbeds a sufficient number of cuttings can be produced for a variety of tests, but additional stools for some clones will be necessary when testing transfers from low to high replication numbers.

Screening Trials

In the context of this paper, a screening trial is defined as the initial clonal evaluation of genotypes where clonal replicates are held to minimal numbers. The split-plot design of the 2010–2011 Screening Trials is comprised of four blocks, five geographic areas, 20-25 clones per area, and planted in two-tree row plots, at a spacing of three by nine feet. In addition to the main plot being the five geographic sources a pseudo sixth source was also created and included all of the sandbar clones regardless of the geographic origin. In 2010, two screening trials were established, with one being located at Stoneville, MS and the second located at Prairie, MS. The Stoneville, MS site included all five geographic areas and 111 clones, of which 99 were black willow clones and 12 were sandbar willow clones. The Prairie, MS site included all five geographic areas but only 82 clones, of which 72 were black willow clones and 10 were sandbar willow clones. In 2011, two additional screening trials were established, with one test located near Hollandale, MS and the second trial located near Prairie, MS. The Hollandale, MS site included all five geographic areas and 111 clones of which 99 were black willow clones and 12 were sandbar clones. The 2011 Prairie site also included all five geographic areas but only 84 clones of which 72 were black willow clones and 10 were sandbar willow clones. Measurements for all four screening trials included a total of eight, and the number of stems at age one, total height, number of stems, and DBH at age two.

Results and Discussion

Since the sampling scheme was initially limited, the decision was made to sample geographic areas that produced genetically superior eastern cottonwood clones for alluvial sites along the lower Mississippi River Valley. In addition, the inclusion of screening trials provides a quick evaluation of all the sampled clones without the need for a large number of cuttings per clone. Elimination of inferior clones during the screening trial stage allows for a more efficient use of land and time. It also allows for additional time to increase ramet numbers per clone which will be needed during the next step. Only the most promising clones will be included in highly replicated clone tests, thus maintaining an efficient test size and the ability to test across a number of sites.

Survival of the 2010 Stoneville test site was excellent averaging 98% at age one and 97% at age two. In comparison, survival of the 2010 Prairie test site averaged 88% at ages one and two. The survival of the 2011 test sites located on the Hollandale site and the Prairie was nearly identical to the 2010 test sites, with the Hollandale site averaging 99% and the Prairie site averaging 88% survival. In both cases, the difference in survival between the two locations was the mortality rate among the sandbar willow at the Prairie site. The one aspect that can be tied to the mortality of the sandbar willow at the Prairie site was the acidic soil pH, which is 4.6. Mortality in the Stoneville and the Hollandale sites was random with no source or clonal pattern being detected. Test site differences were significant for all traits, with height differences accentuated in year two. Test means for the age-one total height, age-two total height, age-2 DBH, and growth between ages one and two for the 2010 Stoneville site was 6.3ft, 13.5ft, 1.1in., and 7.2ft, respectively while the Prairie Site means were was 7.0ft, 11.1ft, 0.7in., and 4.2ft, respectively. The reduction in height growth on the Prairie site was originally attributed to increased weed competition but may also be tied to the acidic soil condition.

Both the separate site analysis and the combined analysis of the 2010 trials indicated significant differences among the five geographic areas and clones within geographic areas. The Atchafalaya River source was the tallest source at age one (7.7ft) and remained the tallest at age two (12.1ft), in the combined analysis. In addition, this source also had the largest age-two diameter (0.81inch.). The performance of the geographic sources showed a general clinal trend. When each test site was analyzed separately, age-one height among the five geographic sources was very similar with the exception of the Trinity River source. The major difference was the Trinity River source, which was the shortest at the end of years one and two at the Stoneville site, but among the tallest at Prairie site for both ages. The faster growing Stoneville site showed a greater range among the geographic sources for height and diameter at age two of approximately 3.0ft. and 0.37in., respectively. The age–two range for height and diameter among sources at the Prairie site is nearly half of that of the Stoneville site at 1.5ft. and 0.18in., respectively.

Significant clonal differences were shown for diameter and height in the combined analysis. The difference between the tallest clone and the test mean was approximately 1.5ft. at age one and increased to nearly 3.0ft. at age two. Four of the top seven clones that were the tallest at age-one remained among the largest at age two. It is also interesting to note that four of the top seven clones that originated from the Atchafalaya River source had the greatest age-two heights and diameters. When the test sites are examined separately, there are greater similarities at age two between the clones that performed well at both 2010 test sites than at age 1. With the exception of age-two height as an indicator over 50% of the clones in the top 10% of the test population originated from the Atchafalaya River. The two clones that remained stable across both test sites at age two were ATCR 2-3 and ATCR 2-5. Other clones that performed well at both sites were ATCR 1-1, BRZ 2-2 and MROS 1-2.

The results of the 2011 Screening Trials revealed that, with the exception of the Trinity River source, the other four sources showed very little difference for age-one height. The Trinity River source, which was significantly shorter than the other four sources in the 2010 tests, showed the same pattern in the 2011 tests. Although the Atchafalaya River source was the tallest at age one, it was not significantly different from the Rosedale, Brazos, and Tunica sources. Clonal performance in the 2011 trials was also similar to the clonal performance of the 2010 trials. Clones such as BRZ 2-2, MROS 1-2, ATCR 2-3, and ATCR 2-5 showed similar rankings in 2011, as well as 2010.

The genetic variability indicated in the 2010-2011 trials showed that considerable gain can be made, especially at the clonal level. The most southern sources produced many of the largest clones through age two. However, a number of fast-growing clones originated from a variety of sources. Although these results are from two-years of growth, the expected rotation for biomass production is three to five years, thus dictating early-age selection. In 2012, all of the available data were used to determine the top 25 clones to be included into a series of highly replicated clone tests. The intent is to establish a series of highly replicated clone tests in 2013 and 2014 that will yield a small number of clones to be recommended for willow biomass plantations in the LMAV. Future work will involve additional collections of native germplasm, creation of new stoolbeds, and the establishment of screening trials.

Summary

It is encouraging that with such a limited initial sampling scheme, the results suggest that significant gains can be made in growth. The extremely high survival rates of black willow are due to excellent rooting characteristics and should greatly aid in biomass yields. Clonal screening trials will provide the best genotypes for further highly replicated clone tests from which a small deployment population will be selected. Additional infusion of new germplasm will broaden the genetic variability and should provide greater gains.

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