DEVELOPMENT OF AN APPLIED BLACK WILLOW TREE IMPROVEMENT PROGRAM FOR BIOMASS PRODUCTION IN THE SOUTH

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ABSTRACT

The development of rapidly growing biomass woody crops is imperative as the United States strives to meet renewable energy goals. The Department of Energy has indicated that biomass is a prime source for renewable energy for the southern United States. Black Willow (*Salix nigra* Marsh.) is a potential bioenergy/biofuels crop for dedicated short-rotation plantations. However, there has been very little genetic development of this species. In 2009, 100 individual one to two year-old whips were selected from five geographic areas and grown in a stool bed near Stoneville, MS. One year-old whips were harvested in February 2010, cut into 14-inch dormant unrooted cuttings, and graded by diameter. The initial study in determining genetic worth is a screening trial where all clones are tested, but with a limited number of ramets per clone. The 2010 black willow screening trial consists of two locations, four blocks, and 100 clones arranged in two tree-row plots at a spacing of 10 x 3 feet. While age-three selections will be used to determine genetic potential, age-one performance will provide insight into clonal performance among the five geographic areas. Selections from the screening trial will be included in highly replicated clone tests. Selections from the screening trial will provide the first genetically improved black willow clones for use in short-rotation plantations.

INTRODUCTION

As the United States moves to increase its production of renewable energy, biomass must play a greater role, especially in the Southeast. Fast-growth tree species, such as poplars (*Populus* spp.) and willows (*Salix* spp.), have been included with energy crops, such as switchgrass (*Panicum virgatum* L.), giant miscanthus (*Miscanthus* x *giganteus*), and energy cane (*Saccharum* spp. L.). Like any crop, tree species grow best when carefully matched to suitable soils. It is important to remember that the production of biomass must be sustainable, must be done with limited inputs, such as fertilizer or irrigation, and must not compete with food sources for land use.

Unlike annual crops, perennial energy grasses, and poplars, black willow (*Salix nigra* Marsh.) grows well on soils that remain wet for long periods during the year. While this type of site is considered marginal for agriculture, it is exactly what is needed to be included in the portfolio of renewable energy options. In essence, black willow biomass production would not remove land from food production and it would utilize sites previously limited in income potential.

Very little research has been undertaken on black willow or willows in general for the southeastern United States, primarily due to its limited economic value. However, the State University of New York College of Environmental Science and Forestry (SUNY-ESF) has had an on-going willow research effort dating back to the 1980s, which has focused on shrub willows, many of which are suited to much colder environments (Abrahamson 2002, Kopp et al. 2001). Since that time, over 20 organizations have formed a Salix Consortium to develop a willow biomass program. Willows represent a viable model for the northeastern and north central United States as a canker disease, (*Septoria musiva* Peck), has severely limited the use of hybrid poplars. Although this program has been successful in the northeastern United States, a willow program has yet to be adopted in other parts of the United States (Volk et. al 2004, Perlack et al. 2005).

Black willow has several key traits that may prove beneficial as a candidate biomass species. These include rapid growth, excellent rootability, ease of vegetative propagation, coppice ability, and exceptional growth on wet sites. Currently, no disease is known that would significantly impact operational use of black willow. Although, cottonwood leaf rust (*Melampsora medusae* Thüm.) will infect willows in the Lower Mississippi Alluvial Valley (LMAV), as it does eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.), the extent of this disease on black willow is an unknown. The ability of black willow to grow and seemingly thrive on poorly drained sites with little impact from diseases is a major reason to look into the development of this species for biomass production. Hybrid poplars have also been discussed as a possibility in the LMAV, but their lack of resistance to *Septoria* canker makes them an impractical choice at this time. Considering all of these factors, black willow seems to be an obvious choice on the heavy clay soils characteristic of poorly drained sites.

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The purpose of this paper is to provide landowners and others interested in growing biomass for bioenergy an initial look into current black willow research. In cooperation with the USDA Forest Service's, Center for Bottomland Hardwoods Research, Mississippi State University has been examining the propagation, silviculture, and genetics of black willow as a viable woody biomass source.

METHOD

The ease of vegetative propagation using dormant unrooted cuttings in *Salix spp.* allows for capture of the total genetic component through the use of clones. But, since black willow is being evaluated as a biomass species, the determination of suitable dimensions of planting stock must precede any other work.

CUTTING LENGTH STUDY

This study was designed to determine the optimal size of dormant unrooted cuttings to be used in experimental trials and operational plantations. It was established in the spring of 2009 on the Mississippi Agricultural Experiment Station (MAFES) near Stoneville, MS. The study design was a 3 x 4 x 3 factorial that included three cutting lengths, four cutting diameters, and three planting depths. Planting material used in this study was harvested from a single geographic source in Oktibbeha Co., MS. Whips were cut into lengths of 9, 15 and 21 inches and separated into diameters of ⅜, ½, ¾, and 1-inch. Planting depth was set by the length of the cutting left out of the ground. Thus, 9-inch cuttings were planted to a depth of 4 and 7 inches, 15-inch cuttings were planted to a depth of 7, 10, and 13 inches, and 21-inch cuttings were planted to a depth of 13, 16, and 19 inches. Prior to planting, the area was disked and sub-soil plowed to a depth of 21 inches. Cuttings were hand planted on a 6 x 10 ft spacing. A broadcast application of Goal® 2XL (oxyfluorfen) was used prior to black willow bud break to reduce herbaceous competition early in the first growing season. For the remainder of the first year, competing vegetation was controlled mechanically at a two-week interval. Canopy closure reduced competing vegetation during the second growing season minimizing the need for weed control. Measurements included total sprout height and basal diameter (one foot above the cutting) at age one, and sprout height and dbh at year two.

GENETIC TRIALS

The initial step in examining the genetic components of black willow is a clonal trial, which is labeled a genetic screening trial and encompasses a total of four sites. Two sites were established in each of 2010 and 2011 using clonal stock from a limited geographic area. The four sites chosen for the Genetic Screening Trial were selected because of their soil and moisture characteristics, which are poorly drained heavy clay sites. These types of sites are exactly where willow is expected to be planted for biomass production. Genetic stock studied in the Genetic Screening Trial was a collection of dormant black willow juvenile seedlings (i.e. called whips) from five different geographic sources in the winter of 2008-2009 (Table 1). The areas selected corresponded to those where some of the historically top performing eastern cottonwood clones originated. From each geographic source, four stands were located and from each of the four stands five individuals were harvested and placed into a stoolbed (i.e. nursery or cutting orchard) during the spring of 2009.

Material for the first two locations of the Genetic Screening Trial planted in 2010 were harvested from the one-year-old stoolbed established in 2009. During the original collection shown in Table 1, some sandbar willow (*Salix interior* Rowlee) was unknowingly collected. This material was maintained in the stoolbed and included in the test. Whips were harvested in the winter of 2010 and cut into 15-inch unrooted cuttings. The experimental design for the Genetic Screening Trial was a split-plot consisting of four blocks, with the main plots within each block designated by geographic origin except for the sandbar clones, which were grouped together. Clones within geographic origin were the sub-plot unit with each clone represented by a two-tree row plot and planted at a spacing of 3 x 10 feet.

The 2010 Genetic Screening Trial included two test sites, one located on the MAFES site near Stoneville, MS and the other located on a MAFES site near Prairie, MS. Site preparation at the Stoneville, MS location included disking and sub-soil plowing on 10-foot centers. Site preparation at the Prairie, MS location included an application of Accord® (glyphosate) at 2 quarts per acre in the summer of 2009, followed by disking, and sub-soil plowing at 10-foot centers. The Stoneville location was planted on April 22, 2010 and included 98 black willow clones and 13 sandbar willow clones. The Prairie, location was planted on April 28, 2010 and included 72 black willow clones and 10 sandbar willow clones. Immediately following the planting of each test location, the area was sprayed with a broadcast application of Goal® 2XL to control herbaceous competition. Survival, total height, and number of shoots were measured at the end of the first growing season.

The 2011 Genetic Screening Trial also included two test sites, one located at the MAFES site near Prairie, MS, and the other on a former rice (*Oryza sativa* L.) field near Hollandale, MS. Site preparation for the Prairie, MS location included disking, and sub-soil plowing at 10-foot centers. Site preparation of the Hollandale location included a chemical application of Roundup ProMax® (glyphosate) and sub-soil plowing at 10-foot centers. The Prairie location was planted on April 25-26, 2011, while the Hollandale, MS location was planted on April 27-28, 2011. Following planting at each location, Goal® 2XL was broadcast applied to control herbaceous competition.

RESULTS AND DISCUSSION

The Cutting Length Study indicated that the longer cuttings tended to result in better age-one and age-two height and that there were no significant age-one and age-two height difference based on cutting diameter. Age-three measurements will complete this study and from that information we will be able to conclude exactly what the optimal dimensions of the dormant unrooted cutting would be for biomass production.

The genetic screening efforts represent a good starting point as over 100 clones from five geographic areas have been included in four field trials over a two-year period. Survival of all four test sites has been good despite very little rainfall during the summer of 2010. However, the sandbar willow tested showed decreased survival at the end of age one when compared to black willow (Table 2). Although not reported here, preliminary rooting studies showed reduced rooting capacity of sandbar willow when compared to black willow. This reduced rooting capacity appeared to manifest itself in mortality in the field trials during moisture stress conditions.

There was very little survival differences among the five black willow geographic sources, with only a two percent range among the sources. This exceptional survival fulfills the first aspect of determining the potential of a biomass species. When used as a dedicated energy crop plantation, high survival rates of planting stock are imperative in maximizing yields. High survival rates are also necessary for possible future coppice regeneration.

Currently, there is no defined rotation length for black willow biomass plantations. However, estimates have suggested that for a black willow dedicated biomass plantation of 1,452 trees per acre, three years may be suitable. These trials will help define age/age correlations and maximum genetic gain per unit of time. During this time frame (i.e. age two) selections will be made and propagated for inclusion into highly replicated clone tests.

Determination of what geographic sources, if any, prove to be superior for the Lower Mississippi River Alluvial Valley would allow for an infusion of new clones into the willow testing scheme. Currently, the 100 plus clones barely scratches the surface of the test population needed to insure that selected clones are genetically superior. To this end, we hope to include additional germplasm into additional screening trials, along with the better clones selected from the first round of testing that will be used as controls. The entire process of clonal infusion, testing, and selection will follow similar processes used in the early stages of *Populus* improvement (Riemenschneider et al. 2001, Robison et al. 2006).

At this point, breeding of improved black willow is not being considered, as the first stage is to simply develop improved clones for operational deployment. But, with a fast-growth species like black willow, hopefully the clonal refinement program can be expanded and accelerated so that breeding may become an integral portion of the program.

SUMMARY

The work described in this manuscript represents an effort to evaluate and develop the potential of black willow as a biomass crop for bioenergy and biofuels. It is imperative to the attainment of renewable energy goals that marginal crop lands sustainably produce biomass crops that can be used for renewable energy. The ability of black willow to grow well on heavy clay sites that have proven difficult for other species makes it a prime candidate for biomass production. However, these sites are not without difficulty, since they are difficult to work due to the high amount of clay and ponding of water that will no doubt affect harvesting, planting, and silviculture practices.

In a little over two years we have been able to establish studies to determine optimal cutting size of unrooted cuttings, genetic screening trials of various geographic origins, stands, and clones, examine rooting architecture, determine that black willow clones are superior to sandbar willow clones on heavy clay sites, and begin to investigate additional propagule types. While the first round of testing has provided some useful guidelines, it has also raised a number of questions that must be answered prior to operational deployment of black willow into dedicated biomass plantations.

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Table 1—Geographic areas, river systems, and coordinates of the black willow cutting collection for the Mississippi State University – USDA Forest Service, Center for Bottomland Hardwoods Research Genetic Screening Trial

Geographic Area	River System	Latitude and Longitude Coordinates
Tunica, MS	Mississippi	34°50'N 90°16'W
Rosedale, MS	Mississippi	32°48'N 91°03'W
Morgan City, LA	Atchafalaya	29°41'N 91°12'W
College Station, TX	Brazos	30°37'N 96°32'W
Liberty, TX	Trinity	30°03'N 94°49'W

Table 2—Age-one survival of the Black Willow Genetic Screening Trial planted in 2010. The table shows comparison between the Prairie and Stoneville locations as well as black willow versus sandbar willow, and geographic sources of black willow

	Age–1 Survival (%)		
Location	Test	Blk vs Sbar[1]	Geographic Sources
Prairie, MS	88		
Black Willow		99	
Sandbar Willow		3	
Geographic Sources			
Tunica, MS			98
Rosedale, MS			100
Morgan City, LA			100
College Station, TX			98
Liberty, TX			100
Stoneville, MS	99		
Black Willow		99	
Sandbar Willow		99	
Geographic Sources			
Tunica, MS			100
Rosedale, MS			99
Morgan City, LA			99
College Station, TX			100
Liberty, TX			99

[1] Blk is the abbreviation for black willow, and Sbar is the abbreviation for sandbar willow.