Breeding, Selection and Testing of Shrub Willow as a Dedicated Energy Crop

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Multiple national imperatives are driving a switch from fossil-based energy sources to renewable energy, including:

- efforts to reduce carbon emissions and slow climate change,
- a push to improve homeland security by reducing reliance on foreign sources of petroleum,
- a desire to stimulate the rural agricultural and forest-based economies, and
- the need to transition from limited fossil fuel resources to sustainable and environmentally benign sources of energy.

Two important components of the portfolio of renewable energy solutions are the conversion of plant biomass to liquid transportation fuels and the production of combined heat and power (CHP) from sustainably produced biomass. Both of these can address the major national imperatives if the production, consolidation and conversion of biomass to either liquid fuel or combined heat and power are accomplished in ways that have highly favorable net returns on energy investment. The production of liquid transportation fuels, such as ethanol, from biomass will provide a transition fuel compatible with much of the current infrastructure and personal vehicle fleet in North America. The use of biomass to produce renewable power can also contribute to reducing reliance on petroleum-based transportation fuels, once plug-in hybrid and electric cars become widely commercially available.

Perennial energy crops will be a major component of overall biomass resources, but there has been little breeding to improve bioenergy traits.

Biomass Feedstocks from Perennial Energy Crops

In considering available and possible biomass feedstocks that could be utilized for the production of biofuels or CHP, there is the potential to collect large amounts of residues from agricultural and forestry sources, as well as low-value biomass from forests (Perlack *et al.*, 2005). However, in order to ensure a long-term and sustainable supply of biomass, there is a need to develop and deploy perennial energy crops on marginal agricultural land specifically to produce biomass for renewable energy projects. These systems would generate multiple societal benefits in addition to producing biomass as a feedstock for fuels and power. Initial research has identified several perennial crops with potential for regional deployment as an energy crop, including a number of perennial grasses, hybrid poplar and shrub willows. Once established, perennial grasses or woody crops can be harvested multiple times over the life of a planting, with relatively low inputs on an annual basis. These plants also tend to accumulate carbon belowground over time and can provide valuable wildlife habitat, while diversifying the agricultural landscape (Volk *et al.*, 2004) In the case of shrub willow, life-cycle assessment indicates that net energy ratios for the production of power by combustion or gasification are in the range of 1:10–15 (Mann and Spath, 1997; Heller *et al.*, 2004).

Shrub Willow as a Dedicated Energy Crop

Shrub willow has been developed as a dedicated energy crop since the mid-1970s, when researchers in Sweden (Christersson *et al.*, 1993), and not long after in Canada and the United Kingdom, recognized the potential of this fast-growing plant that vigorously resprouts the spring after the stem biomass is harvested (coppiced). Willow typically breaks bud very early in the season and can have a high leaf-area index, thus can be very efficient in capturing available seasonal irradiation. Although willow species are often found in wetlands, along creeks, and in other flooded habitats and can tolerate poorly drained soils, they can also thrive in upland fields and grow very fast in moderately well drained soils that receive regular rainfall (Newsholme, 1992). In this respect, shrub willows can be planted on otherwise marginal agricultural soils that do not support high yields of corn or soybean due to poor drainage conditions, limited fertility, or regular spring flooding.

Willow is planted by pushing a section of dormant 1-year-old stem into the soil of a properly tilled field, after which it will produce roots and the dormant buds will emerge to form new stems. Willow fields are planted in a double-row arrangement at ~15,000 plants per hectare (ha^{-1}), with 0.76 m between rows, 0.61 m between plants in a row, and 1.52 m spacing between double rows to allow clearance for cultivation and harvesting machinery. Planting can be accomplished using a four- or six-row planter attachment to a tractor, which accepts >2 m-long whips and cuts them into 20-cm sections (cuttings). Pre-emergent herbicides are applied soon after planting to control weeds (Kopp *et al.*, 1992), which is critical for successful plantation establishment. Fall site preparation and planting of a winter cover crop are advisable on soils with a higher soil erosion potential (Volk, 2002). If weeds do become problematic, especially in the first 2 years, mechanical cultivation can be applied between the rows. At the end of the establishment year, plants are typically coppiced, which stimulates new growth the following spring. From that point on, the plants are harvested every 3 or 4 years for seven or more rotations. Harvesting can be accomplished using a self-propelled forage harvester equipped with a specialized or modified cutter head capable of sawing the stems just above the soil and feeding them into the harvester, which delivers wood chips—uniformly 5 cm or less in size—to a wagon or truck. The window for harvesting opens immediately after senescence and leaf fall, and continues until bud break in the spring, which allows nutrients to be recycled to the root system and may be done when the ground is frozen to reduce soil compaction and rutting. Chips are trucked to the fuel yard of the power plant and are piled for storage and moderate drying before conversion by combustion or gasification. Typically, a modest amount of slow-release fertilizer (100 kg N ha^{-1}) is added in the spring after each harvest (Adegbidi *et al.*, 2003).

Genetic Improvement of Shrub Willow and Selection of Varieties for Bioenergy Traits

Early commercial-scale demonstration of shrub willow bioenergy crops in the United States relied on varieties developed in the breeding program of Louis Zsuffa at the University of Toronto that had been tested in trials at SUNY-ESF. Many of these varieties were F_1 progeny of crosses of *Salix eriocephala*, and a number of these were moderately or severely susceptible to *Melampsora* spp. rust. Varieties developed in Sweden (Larsson, 2001), and deployed commercially by Svalöf Weibull AB (now Lantmännen Agroenergi), were tested in New York and quickly found to be susceptible to damage by potato leaf hopper (R.F. Kopp and L.P. Abrahamson, unpublished). Thus, in order to develop new varieties with improved yield and to support the long-term deployment of shrub willow crops in North America, SUNY-ESF initiated a willow-breeding program in the mid-1990s. Since 1994, a diverse collection of more than 700 willow accessions, representing over twenty species and hybrids, has been assembled through collection of naturally established plants in the wild or disturbed environments, contributions of naturally collected or bred germplasm from United States and overseas collaborators, and from the purchase of varieties available from commercial nurseries (Smart *et al.*, 2005). Techniques for the collection of pollen and for mechanical pollination were developed and adapted for the species in the breeding program (Kopp *et al.*, 2002). Since 1998, researchers at SUNY-ESF have produced approximately 200 families from more than 575 attempted controlled pollinations.

Selection and testing of clones has been accomplished through three levels of field trials:

- family screening trials,
- selection trials, and
- regional yield trials.

Crosses were completed in 1998 and a family screening trial was established in the field at LaFayette Road Experiment Station in Syracuse, NY, but due to a facilities-management decision, this trial was removed in 1999 and selections were made based only on preliminary growth evaluations. Thirty individuals were selected and propagated in nursery beds for 2 years to generate sufficient cuttings to establish a replicated selection trial in 2001 consisting of sixteen of those clones, as well as four individuals collected from natural stands, and five reference varieties, some of which were used as parents in the 1998 crosses. Crosses completed in 1999 produced forty-six families that were evaluated in a family screening trial in the field at LaFayette Road Experiment Station. More than 2,000 seedlings were planted in linear plots by family with 0.3-m spacing between plants and 1 m between rows. The seedlings were coppiced after the first season and then stem height, number of stems, and diameters were measured after two seasons of growth. Based on those measurements, four families were chosen as having superior overall family performance and the top fifteen individuals were selected from each family. A total of twenty-two other exceptional individuals were selected from eight other families. Cuttings were made from these plants for the establishment of a replicated selection trial in 2002.

The 2001 selection trial was planted at the Tully Genetics Field Station in Tully, NY, using dormant 25-cm cuttings in typical production spacing. Each plot contained forty plants (twenty plants per row with one double-row per plot) and was replicated in three completely randomized blocks. These plants were coppiced at the end of the first growing season, then were subsequently harvested after three growing seasons post-coppice (end of 2004). The innermost twenty plants per plot were weighed and subsamples were collected and dried to determine moisture content, so that total dry biomass could be calculated per plot. Based on these first-rotation harvests, nine of sixteen clones produced through breeding yielded greater mean biomass than the reference variety *S. dasyclados* 'SV1', which had a mean yield of 7.4 oven-dry tons (odt) ha^{-1} yr^{-1} (Fig. 1). The top variety in this trial after one harvest rotation was *S. miyabeana* 'SX64', with mean yield of 11.3 odt ha^{-1} yr^{-1}, 53% higher than that of 'SV1'.

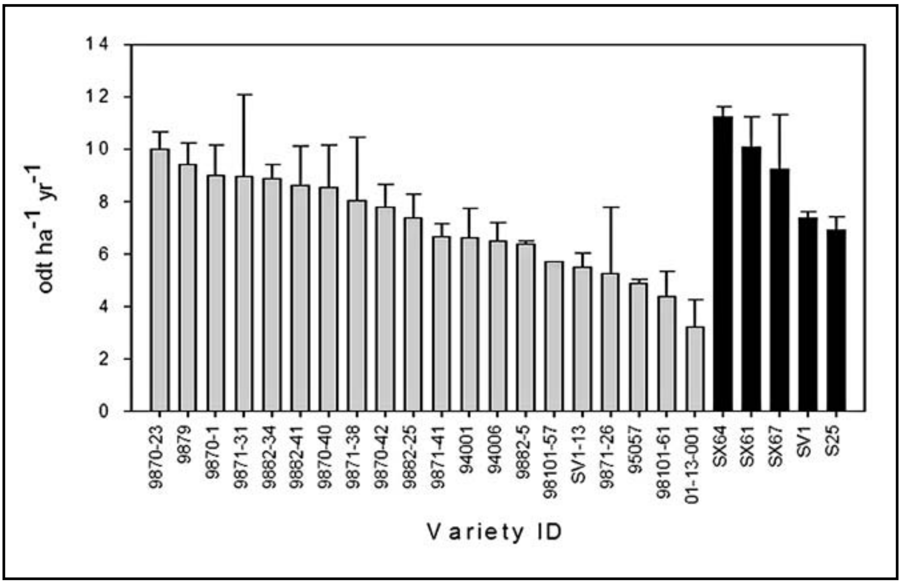

Figure 1. Mean first-rotation production of varieties tested in the 2001 genetic selection trial at Tully, NY. Grey bars (± standard error) represent varieties produced through controlled breeding or collected from naturally established stands. Black bars represent current production varieties for reference.

The 2002 selection trial was planted at the Tully Genetics Field Station using rooted 12- to 17-cm cuttings in four-plant row plots with 0.6 m between plants in a row and 0.9 m between rows (~18,500 plants ha^{-1}). Each four-plant plot was replicated in eight completely randomized blocks, each of which contained eighty-two new clones and four reference varieties. Some plots suffered mortality soon after planting, most likely due to exposure and sensitivity of the roots to herbicide that had been applied at planting time, since there has been little further mortality after year 1. These plants were coppiced at the end of the first growing season, then stem height, number, and diameters of the inner two plants per plot were measured at the end of the first growing season post-coppice (end of 2003). Based on calculations of total stem area per plant after one growing season, sixty-nine of eighty-two new varieties produced greater total stem area per plant than the reference variety 'SV1' (Fig. 2). The mean total stem area of the top clone (99202-011) was 114% greater than that of 'SV1'. Based on these measurements, cuttings were made from 1-year-old stems of forty-two of the original family screening trial plants and planted in nursery beds to scale-up for future trials. First-rotation harvest of the 2002 selection trial was completed after the second growing season post-coppice (end of 2004) and a second harvest was done 2 years later (end of 2006). To obtain an estimate of growth potential and account for the anomalous establishment mortality, measurements of plots with less than three living plants were removed from the data set. A modest amount of fertilizer (100 kg N ha^{-1}) was applied in the spring after the first harvest. Based on yields from each harvest, twenty-four of the new clones and variety 'SX64' produced greater dry biomass than reference variety 'SV1', which produced 11.9 odt ha^{-1} yr^{-1} in the second

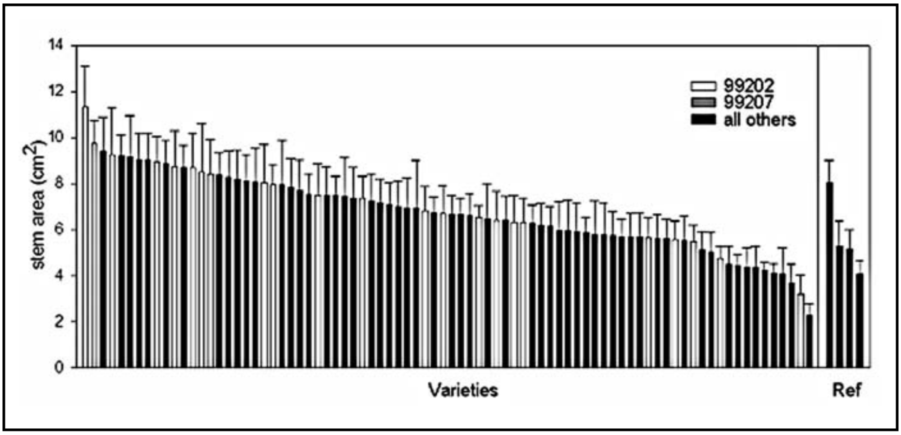

Figure 2. Mean stem area per plant (± standard error) of varieties tested in the 2002 genetic selection trial, Tully, NY. Stems larger than 3 mm were measured at a height of 30 cm at the end of the first growing season post-coppice. The four bars on the right represent current production varieties for reference. Open bars represent siblings in family 99202, grey bars siblings in family 99207, and black bars members of several other families.

2-year harvest rotation of this trial. The top clone (99202-011) produced a mean biomass yield of 21.9 odt ha^{-1} yr^{-1} in these small experimental plots. Overall mean yields increased 6.2% from first harvest to second, and sixty of the eighty-six clones in the trial produced greater yields in the second rotation. Although these yields are impressive, they were produced in very small plots on a single site. To test the potential yield in commercial-style plantings and plasticity to varying site conditions, it is necessary to test these clones at many varied sites in larger plantings.

Prior to large-scale commercial deployment, the best estimate of variety responses to regional environmental differences and soil types is determined through replicated yield trials with multiple varieties planted in commercial-style spacing. Based on measurements and harvest yields from the 2001 and 2002 selection trials, varieties were selected to be planted in yield trials that were established in Belleville and Tully, NY, in 2005. These trials contain fourteen new varieties and four reference varieties planted by hand with 25-cm dormant cuttings in seventy-eight-plant plots arranged in three double-rows that each have thirteen plants per row. Each trial contains four complete randomized blocks, for a total of 312 plants per variety and 5,616 plants overall. These trials were coppiced at the end of the establishment-year growing season (end of 2005). At the end of the 2006 growing season (first-year post-coppice), stem diameters, number, and height of the tallest stem were measured for the inner eighteen plants of the middle double-row. Together, stem diameter and stem number are reliable predictors of biomass yield, but they do not account for differential biomass density and second- and third-season differences in growth among varieties (Tharakan *et al.*, 2001, 2005). Comparisons of the first-year post-coppice stem-area measurements of these two trials highlight the potential for genotype-by-site interactions. Among the reference varieties, *S. sachalinensis* 'SX61' produced the greatest mean stem area per plot and *S. miyabeana* 'SX64' was ranked 4th at Tully, while 'SX61' was ranked 13th and 'SX64' was 18th in the Belleville trial. In contrast, reference variety *S. eriocephala* 'S25' was ranked 14th and *S. dasyclados* 'SV1' was 16th at Tully, but 'S25' was 4th and 'SV1' was 2nd at Belleville. Among the new varieties, the mean stem area per plant for 99239-015 and 9871-31 were significantly greater than that of reference variety 'SV1' over both sites combined. At least with respect to total stem area per plant measured in these trials, the current production varieties 'SV1,' 'S25,' 'SX61,' and 'SX64' display site-specific patterns of growth productivity. A positive outcome of this testing will be to identify new varieties that display high yield and greater plasticity to site and environment conditions, so that growers need not be overly concerned about matching specific varieties to particular combinations of site characteristics.

One of the major bottlenecks to widespread commercial deployment of new perennial energy crops is the scale-up of high-quality planting stock, whether it is seed lots of switchgrass, rhizomes of *Miscanthus x giganteus*, or whips of shrub willow. Considering the urgency of the need to dramatically expand acreage planted with energy crops to meet national goals, the selection and propagation of improved varieties must occur as soon as possible. To begin to break this bottleneck, SUNY-ESF and the Research Foundation of SUNY have licensed shrub-willow varieties developed through research at the College to Double A Vineyards dba Double A Willow (www.DoubleAWillow.com) for production and commercial sale of willow planting stock (whips, stakes and cuttings). Since willow stems have very weak maintenance of bud dormancy, it is critical to store dormant stems frozen at –2 to –4°C, which requires capital investment in large volumes of storage freezer capacity at any shrub willow nursery. With funding from the New York State Energy Research and Development Authority, Double A Willow has installed storage freezers capable of holding approximately 10 million cutting equivalents. They have also established nursery beds with over 100,000 plants currently representing sixteen biomass varieties, with plans to dramatically expand those this year and in the future. With this type of commercial development and investment, there is good possibility that cultivation of shrub willow crops will expand to help meet the needs of society for renewable and sustainable sources of energy.

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Smart received his PhD in genetics at Michigan State University in 1992 and was an NSF postdoctoral fellow at the University of California-Davis. Since 1996, he has taught courses in cell physiology, plant physiology, techniques in plant physiology, and a senior synthesis in biotechnology at SUNY-ESF. In addition to *Salix* genetics, Dr. Smart's research includes studies of cuticular wax biochemistry, stomatal physiology, and drought tolerance.