A mixed model approach for evaluating yield improvements in interspecific hybrids of shrub willow, a dedicated bioenergy crop

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A B S T R A C T

Lignocellulosic bioenergy crops such as shrub willow (*Salix* spp.) are expected to have a significant role in climate mitigation strategies. Yield is perhaps the most important trait for genetic selection and development of biomass feedstocks, but the selection process is complicated by the strong influence that environmental factors has on cultivar performance. Understanding the genotype-by-environment interactions using regional yield trials of shrub willow will inform future breeding efforts and improve the ability to make regionally-specific cultivar recommendations. We analyzed two yield trial datasets containing genotypes from successive rounds of breeding using a series of mixed models. Stability variance parameters were used together with overall yields to identify genotypes with stable yields or specific adaptation. Analysis of the first dataset revealed a group of triploid interspecific hybrid cultivars with exceptional performance and specific adaptation to either poor or good quality environments. The best performing cultivar showed a 14% yield improvement over the mean of the check cultivars. In the second dataset, new selections were assessed along with the top performing cultivars from dataset 1. New triploid hybrids had yields 19 to 27% greater than the check cultivar and exhibited similar patterns of specific adaptation as some of the selections from the first dataset. Partial least squares regression analysis indicated that top-yielding genotypes were favored in environments with higher temperatures and greater growing degree days. This analysis demonstrates incremental improvements in yield with successive rounds of breeding through the development of interspecific triploid hybrids.

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1. Introduction

Increasing energy production from renewable, low-carbon sources is an imperative for mitigating the impacts of climate change. Current US national energy policy mandates a rapid expansion of cellulosic biofuel production over the coming years (Environmental Protection Agency, 2007). These statutory provisions will likely only be met by increasing the area devoted to growing dedicated bioenergy crops, especially on low productivity agricultural lands. Perennial lignocellulosic feedstocks such as shrub willow (*Salix* spp.) and poplar (*Populus* spp.) managed in short rotation coppice systems are expected to provide a substantial portion of biomass needed in the US to achieve renewable energy goals

(U. S. Department of Energy, 2011). These crops remain on the landscape for many years, so selecting cultivars that are not well matched to local climatic and edaphic conditions can be a costly mistake to commercial growers.

Shrub willow is a fast-growing, woody perennial C_3 feedstock that is well adapted to humid, temperate regions such as the northeastern and upper midwestern US. It is typically managed on 2–4 year harvest cycles with an expected productive crop life of over 25 years. In addition to supplying large amounts of biomass over short periods of time, shrub willow has low or net negative greenhouse gas emissions (Caputo et al., 2014; Djomo et al., 2015; Wightman et al., 2015), mainly through high rates of C storage in belowground components (Cunniff et al., 2015; Pacaldo et al., 2013, 2014), and has greenhouse gas mitigation potential when converted to bioenergy for displacement of fossil fuel (Djomo et al., 2011). Other features that make shrub willow interesting for breeding are that it is dioecious, is amenable to interspecific

hybridization, has multiple ploidy levels, and can be clonally propagated. Shrub willow re-sprouts vigorously after harvest. has a low nutrient demand and high nutrient use efficiency (Aronsson et al., 2014; Hangs et al., 2014) and a large potential for applications in phytoremediation projects (Kuzovkina and Quigley, 2005).

In the US, commercial production of shrub willow for use in biopower production was recently initiated on a small scale in New York State, in part due to a regionally specific federal assistance program (Volk et al., 2016). In Canada, despite decades of research into the production potential of shrub willow, particularly of native species, wide-spread adoption has been hampered by socio-economic issues (Larocque et al., 2013; McKenney et al., 2014). Current economic analyses of short-rotation biomass production including willow suggest that yield is an important factor determining economic viability (Buchholz and Volk 2011; Hauk et al., 2014). Recent breeding efforts in the US have focused largely on improving yields by capturing heterosis from novel interspecific crosses (Smart and Cameron 2012; Smart et al., 2005). Specifically, crosses between diploid and tetraploid species producing triploid progeny have demonstrated substantial yield gains over commercial check cultivars in early stages of evaluation (Serapiglia et al., 2014).

Yield data on short rotation woody crop production in North America is somewhat limited, due largely to its relatively recent reemergence on the continent as a biomass feedstock (Volk et al., 2006). However, recent initiatives aimed at testing newly bred cultivars in regional yield trials have generated valuable datasets for testing genotype-by-environment interactions (GEI) in North America (Serapiglia et al., 2014; Volk et al., 2011). Traditional analysis of variance (ANOVA) approaches to multi-environment datasets have demonstrated significant GEI in shrub willow (Larsen et al., 2014; Mosseler et al., 2014; Serapiglia et al., 2013). However, these approaches often only test for the presence or absence of statistically significant interactions and thus do little to describe potential structure in the interaction, through which genotype stability or adaptability can be observed. Understanding these nuances is of great importance to breeding programs. Popular approaches to exploring yield stability include regression on environment mean techniques (Eberhart and Russel 1966; Finlay and Wilkinson, 1963) or partitioning the total GEI variance among individual contributions from each genotype (Shukla, 1972). While the regression approach is intuitive, it is often criticized for capturing two little of the GEI variance (Crossa, 1990). Gollob (1968) suggested combining ANOVA with factor analytic decomposition of the interaction matrix in order to reduce noise and find structure among the interacting factors. Gauch (1988) popularized this approach for crop yield trial data with the so-called Additive Main Effects and Multiplicative Interactions (AMMI) model. The AMMI model is a powerful technique for interaction noise reduction, resulting in the assignment of interaction scores to each genotypes which are accurate measures of yield stability or adaptability. In a recent analysis of shrub willow yield trials across North America, Fabio et al. (2016) used the AMMI model to confirm the superior performance of a group of triploid hybrids, which were identified as having greater yields and exhibiting differential patterns of broad and narrow adaptability. That evaluation was restricted, however, to 16 genotypes present in 10 environments. This restriction was imposed in order to satisfy the need of a balanced dataset when using fixed-effects AMMI.

More recent efforts have demonstrated the utility of mixed effects models for the analysis of GEI datasets because they offer a flexible framework for exploring the GEI, specifically through the application of different variance-covariance structures, which impose certain assumptions about the complexity of the random factor variances (Hu and Spilke 2011; Sixto et al., 2014). The mixed model approach can handle unbalanced datasets, which are common in yield trial evaluations and can also incorporate heterogeneity of variances at multiple levels (Piepho et al., 2003; Smith et al., 2005; Raman et al., 2011).

In this study we present first rotation data from two sets of yield trials representing early and more recent stages of genetic improvement in shrub willow. Because not all genotypes evaluated were present in every test environment, the mixed model approach allowed us to incorporate a much greater number of observations available in the datasets than would otherwise allow in a fixed-effects framework. The objectives were (1) to test a series of mixed models on an existing dataset of shrub willow yields (Dataset 1) to identify stable and regionally adapted cultivars, (2) to analyze a new dataset (Dataset 2) of improved genotypes to identify yield improvements after successive breeding and selection and (3) to use environmental covariates to explain patterns in genotypic sensitivities to growing conditions in both datasets.

2. Materials and methods

2.1. Yield trial dataset 1

Breeding material consisted of native and naturalized accessions of *Salix eriocephala* and *S. purpurea*, collected from the northeastern US and eastern Canada, in combination with germplasm secured from the University of Toronto, consisting mainly of improved *S. eriocephala* and natural collections of *S. miyabeana*, native to East Asia, as well as *S. viminalis*, a native of Europe. Controlled intraspecific and interspecific crosses were performed at the State University of New York College of Environmental Science and Forestry, mostly in 1998 and 1999. Progeny from each successful cross were planted in nursery beds and individuals were selected based on growth traits for evaluation in a replicated selection field trial in Tully, New York in 2002 (Smart et al., 2008). Top-performing genotypes across multiple pedigrees were advanced to multi-location yield trials (Table 1). These yield trials were established between 2005 and 2011 and were hosted by eight institutions across six US states and two Canadian Provinces (Table 2). For detailed descriptions of trial establishment and design see Serapiglia et al. (2013) and Fabio et al. (2016). Planting material for each trial originated as 25-cm dormant, hardwood cuttings sourced from nursery beds at the SUNY-ESF Genetics Field Station in Tully, NY. After conventional site preparation, cuttings were planted by hand, usually in May or June, in a double-row configuration at a planting density of approximately 14,400 plants ha^{-1}. The distance between plants within a row was 0.61 m, between adjacent rows 0.76 m, and between double rows 1.52 m (alleys). Experimental units consisted of plots planted with a single cultivar, three double rows wide and 13 plants long. The outer two double rows were considered guard rows and only the middle double row was used for measurements in order to avoid edge effects. Each trial was planted with between 18 and 30 genotypes in a randomized complete block design, with four replicates of each genotype. Three check cultivars, 'SV1′, 'SX61′ and 'SX64′ were used throughout testing and at least two checks were present in each trial.

After the first year of growth all aboveground biomass was cutback during dormancy close to ground level to promote a multi-stem coppice regrowth response in the following spring. At that time most trials received a single application of 112 kg N ha^{-1} applied as ammonium sulfate. None of the Michigan trials and the trial in Saskatchewan received fertilizer during the study period. Trials were allowed to grow for three years after cutback, at which time 18–22 plants from the middle double row of each plot were harvested. Harvests were performed either manually by cutting whole stems at ground level with a brush saw and weighed with spring scales, or mechanically in a single-pass cut and chip oper-