3-2017

Biomass Yield of Switchgrass Cultivars under High- versus Low-Input Conditions

Michael D. Casler
USDA-ARS

Sergio Sosa

Lindsey Hoffman
Rutgers University - New Brunswick/Piscataway

Hilary Mayton
Cornell University

Calvin Ernst

See next page for additional authors

Authors
Michael D. Casler, Sergio Sosa, Lindsey Hoffman, Hilary Mayton, Calvin Ernst, Paul R. Adler, Arvid R. Boe, and Stacy A. Bonos

Published February 23, 2017

RESEARCH

Biomass Yield of Switchgrass Cultivars under High- versus Low-Input Conditions

Michael D. Casler,* Sergio Sosa, Lindsey Hoffman, Hilary Mayton, Calvin Ernst, Paul R. Adler, Arvid R. Boe, and Stacy A. Bonos

ABSTRACT

Switchgrass (*Panicum virgatum* L.) is undergoing development as a biomass crop to support conversion of cellulosic biomass to energy. To avoid the competition of biomass with food or feed crops, most commercialization proposals suggest that switchgrass should be grown exclusively on marginal lands that are not fit for food or feed production. The objective of this study was to investigate the potential for cultivar × environment interactions that would affect the methods and approaches for breeding and evaluating switchgrass cultivars, including both upland and lowland types, for high-input versus low-input types of environments. Biomass yield was measured on 14 cultivars that were present in 28 replicated field experiments representing seven regions, ranging from 75 to 100° W and spanning USDA Hardiness Zones 4 through 7. Region was the most important environmental factor interacting with cultivars, supporting the idea that the north-central and northeastern United States should have independent switchgrass breeding programs. Cultivars interacted with soil phosphorus concentration in New Jersey and with depth of the A and B horizons in New York and showed mild interactions with rate of nitrogen fertilizer at several locations. Cultivar rank correlation coefficients between the two rates of nitrogen fertilization (100 vs. 0 kg N ha^{-1}) ranged from 0.23 to 0.88, suggesting a possible benefit to breeding and selection without applied nitrogen fertilizer.

M.D. Casler, USDA-ARS, 1925 Linden Dr., Madison, WI 53706-1108; S. Sosa, 294 Lamplighter Ln., Marietta, GA 30067; L. Hoffman, Rutgers Univ., Dep. of Plant Biology and Pathology, 59 Dudley Rd., Foran Hall, New Brunswick, NJ 08901; H. Mayton, Cornell Univ., Plant Pathology and Plant-Microbe Biology Section, 356 Plant Science, Tower Road, Ithaca, NY 14853; C. Ernst, Ernst Conservation Seeds, 8884 Mercer Pike, Meadville, PA 16335; P.R. Adler, USDA-ARS, Pasture Systems and Watershed Management Research Unit, University Park, Pennsylvania 16802; A.R. Boe, South Dakota State Univ., Dep. of Plant Science, Agricultural Hall, Brookings, SD 57007; S.A. Bonos, Rutgers Univ., Dep. of Plant Biology and Pathology, 59 Dudley Rd., Foran Hall, New Brunswick, NJ 08901. Received 23 Aug. 2016. Accepted 29 Dec. 2016. *Corresponding author (mdcasler@wisc.edu, michael.casler@ars.usda.gov). Assigned to Associate Editor Hem Bhandari.

Abbreviations: GE, genotype × environment; HYE, high-yield environment; LYE, low-yield environment; PLS, pure live seed.

NUMEROUS perennial grass species are undergoing intensive research and development as dedicated bioenergy feedstocks. Switchgrass (*Panicum virgatum* L.) is one of the more prominent and visible of these species, having been chosen by the US Department of Energy as its model herbaceous feedstock (Sanderson et al., 2006). Biomass yield is the principal limitation to economically viable and sustainable biomass production from switchgrass, particularly in the northern United States, where growing seasons are short (Perrin et al., 2008). Early efforts to improve biomass yield of switchgrass focused on long-term, field-based selection and breeding combined with agronomic experiments to determine optimal adaptation regions for existing cultivars (Casler et al., 2004, 2007; Casler and Vogel, 2014). More recent efforts have focused on increasing the breadth of germplasm collection and selection for winter survival within late-flowering populations, which are designed to extend the biomass growth cycle through the end of the growing season (Casler, 2014; Casler and Vogel, 2014). Together, these efforts have increased

Published in Crop Sci. 57:821–832 (2017).
doi: 10.2135/cropsci2016.08.0698

biomass yield by ~50% in northern climates with optimal growing conditions (Casler, 2014; Casler and Vogel, 2014).

A considerable amount of selection and breeding of switchgrass, as well as some agronomic research, is conducted on public agricultural experiment station lands, which are often sited on prime farmland (McLaughlin and Kzsos, 2005; Parrish and Fike, 2005; Casler et al., 2012). In addition, it is common practice to apply nitrogen (N) fertilizer to switchgrass breeding nurseries and research plots, usually in the range of 40 to 100 kg N ha^{-1} (Casler et al., 2012). Levels of N fertilizer are designed to replace N removed in the previous season's biomass crop but are not so high as to lead to leaching from the soil (Vogel et al., 2002). Nevertheless, the practice of growing switchgrass for biomass on prime farmland with N fertilizer is at odds with the growing philosophy toward low-input and sustainable biomass production that does not compete with agriculture for human food (Gopalakrishnan et al., 2011; Mitchell et al., 2012; Gelfand et al., 2013).

World population growth and global climate change are placing more pressure on agriculture to meet human nutritional needs, which in turn places pressure on the bioenergy industry to produce biomass without displacing food or feed production (Kang et al., 2013; Shortall, 2013). While there are various definitions of marginal lands, and land can be "marginal" for many reasons, one universal definition can be generalized as land that fails to meet local minimum thresholds for economic production of food or feed crops (Shortall, 2013; Richards et al., 2014). With appropriate policies, incentives, and infrastructures, some lands that are marginal for food or feed production could be used for biomass production (Kang et al., 2013; Shortall, 2013; Milbrandt et al., 2014). For example, proposals have been advanced to produce biomass on nonirrigated pivot corners (Uden et al., 2013), reclaimed surface mines (Brown et al., 2016), and buffer strips surrounding sensitive surface waters (Hernandez-Santana et al., 2013; Porter et al., 2015), all within regions where food and feed are produced on prime farmland.

Development of dedicated and sustainable biomass crops will require efficient plant breeding, cultivar evaluation, and agronomic production systems. Optimally, these systems should be developed and deployed on lands representative of those where the biomass crops will be grown (Brummer et al., 2011; Brummer and Casler, 2014). Switchgrass is highly sensitive to genotype × environment (GE) interactions, in which cultivar rankings vary widely under differing environmental conditions (Casler et al., 2004, 2007, 2012). Temperature, photoperiod, and moisture availability are all important drivers of GE interactions in switchgrass, but little to nothing is known about the role of soil conditions in driving GE interactions in switchgrass. Rose et al. (2007) showed that genetic improvement in biomass yield was greater under low-yield environments (LYEs) versus high-yield environments (HYEs), where the environmental difference was due to a combination of irrigation and fertilization with N, phosphorus (P), and potassium versus none of these inputs.

In the current study, we investigate GE interactions of switchgrass cultivars, specifically in response to two factors: soil quality and N fertilizer. Our experiments were conducted across a broad landscape, spanning the northern United States from the 75th to the 100th meridian and a wide range of soil types. Specifically, our objective was to determine the relative importance of GE interactions for switchgrass cultivars where the environment is divided into three independent factors: geographic region, soil quality within region, and N fertilizer within soil quality and region. The central question was: are there substantial changes in the ranking of cultivars for biomass yield under different soil quality or N fertilization conditions? More specifically, is there any danger in missing the target of improving production on marginal soils if breeding is conducted on prime soils?

MATERIALS AND METHODS

Field experiments were planted in seven regions in April or May 2008 or 2009: Maryland, New Jersey, New York, central Pennsylvania, northwestern Pennsylvania, South Dakota, and Wisconsin (Table 1). Several experiments failed to establish in 2008, and these were replanted in 2009 (Table 1). Two soil quality categories were chosen within each region, meant to represent prime and marginal farmland. Because the definition of prime versus marginal farmland varies widely (Richards et al., 2014), there were several defining characteristics used to make the distinction between prime and marginal sites (Table 1). In all cases, it should be stressed that these were hypothetical designations—we hypothesized that each of the prime versus marginal designations would have an impact on adaptation and performance of switchgrass but did not have any definitive a priori knowledge of this effect. Two regions were based on similar soils that differed in presence or absence of a fragipan (two Pennsylvania regions). The New York sites differed in depth of the A and B horizons. The Maryland sites differed in drainage due to differential soil type. The New Jersey sites differed slightly in the depth of the A horizon and A-horizon fertility but had a large difference in P concentration. One region was based on clay content and depth to bedrock (South Dakota). Lastly, one region (Wisconsin) was based on pH differences, established over 30 yr of continuous maize (*Zea mays* L.) production, confirmed by soil tests.

Within the 14 sites defined by regions and soil categories (Table 1), two experiments were planted, one to be treated with N fertilizer and one to be kept unfertilized. Each of the 28 experiments was designed as a randomized complete block with three replicates (or four replicates for the Hancock, WI, experiments). Plot size varied across locations, according to available equipment (Table 1). Fourteen cultivars were included in each experiment (Table 2). Seed for all cultivars was stratified by Ernst Conservation Seed 60 d prior to planting, according to Shen et al. (2001), and germination tests were conducted by the Ohio Seed Improvement Association in the winter 2008–2009 to determine pure live seed (PLS) seeding rates. Seeding rates of all cultivars were standardized to ~6 kg PLS ha^{-1}. Switchgrass seeds were also treated with Raxil (tebuconazole + triflumuron), Thiram (tetramethylthiuram disulfide), and Poncho (clothianidin) to improve germination. Plots were planted either by broadcasting seed (New Jersey) and cultipacking (Maryland) or in drill rows with 15-cm spacing (all other regions). All locations were planted during optimum switchgrass germination periods during the late spring to early summer of 2008 and/or 2009. No herbicides were used during the establishment year.

Plots were allowed to grow without clipping during the establishment year. Prior to initiation of growth in spring of the first production year, half of the field experiments, as predetermined, were fertilized with 100 kg N ha^{-1}. Biomass yield was harvested a single time for each experiment, shortly before or after killing frost. Biomass was harvested using either a flail chopper (Wisconsin and South Dakota) or a sickle-bar harvester

Table 1. Characteristics of the seven regions used to evaluate switchgrass cultivars.

Location and establishment year	Soil category	Prime vs. marginal defining characteristic	Soil series and taxonomy	Latitude	Longitude	HZ†	Plot size‡
				° N	° W		m
Snow Hill, MD 2009	Prime	Well-drained, sandy loam	Sassafras sandy loam (Fine-loamy, siliceous, semiactive, mesic Typic Hapludults)	38.22	75.38	7b	1.7 × 1.8 (0.9 × 1.8)
Snow Hill, MD 2009	Marginal	Poorly drained, low area	Othello silt loam (Fine-silty, mixed, active, mesic Typic Endoaquults)	38.22	75.38	7b	1.7 × 1.8 (0.9 × 1.8)
Adelphia, NJ 2009	Prime	High P (400–700 mg kg^{-1}); 27-cm A horizon	Freehold sandy loam soil (fine-loamy, mixed, active, mesic Typic Hapludults)	40.23	74.25	7a	1.8 × 1.8 (0.9 × 1.8)
Somerset, NJ 2009	Marginal	Low P (30–60 mg kg^{-1}); 20-cm A horizon	Klinesville loam (loamy-skeletal, mixed, active mesic Lithic Dystrudepts)	40.47	74.53	7a	1.8 × 1.8 (0.9 × 1.8)
Ithaca, NY 2009	Prime	AB horizons 60 cm; no fragipan	Niagara silt loam (fine-silty, mixed, mesic Aeric Ochraqualfs)	42.45	76.45	5b	1.1 × 3.6 (0.9 × 3.6)
Ithaca, NY 2008	Marginal	AB horizons 30 cm; fragipan	Langford silt loam (fine-loamy, mixed, mesic Typic Fragiochrepts)	42.47	76.44	5b	1.1 × 3.6 (0.9 × 3.6)
Rock Springs, PA 2008	Prime	AB horizons 90 cm; no fragipan	Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs)	40.72	77.94	6b	1.9 × 3.0 (1.5 × 3.0)
Rock Springs, PA 2008	Marginal	Poorly drained; depth to fragipan ~60 cm	Andover silt loam (fine-loamy, mixed, mesic Typic Fragiaquults)	40.70	77.95	6b	1.9 × 3.0 (1.5 × 3.0)
Rockton, PA 2010	Prime	AB horizons 90 cm; no fragipan	Clymer loam (coarse-loamy, siliceous, active, mesic Typic Hapludults)	41.12	78.65	5b	1.9 × 3.0 (1.5 × 3.0)
Rockton, PA 2010	Marginal	Poorly drained; depth to fragipan ~20 cm	Brinkerton silt loam (fine-silty, mixed, superactive, mesic Typic Fragiaqualfs)	41.12	78.65	5b	1.9 × 3.0 (1.5 × 3.0)
Aurora, SD 2008	Prime	Well-drained and deep silty clay	Brandt silty clay (fine-silty, mixed, mesic, superactive, frigid Calcic Hapludolls)	44.30	96.67	4b	0.9 × 6.1 (0.9 × 6.1)
Pierre, SD 2008	Marginal	Depth to bedrock ~80 cm; heavy clay	Opal clay (fine, smectitic, mesic Leptic Haplusterts)	44.36	100.00	4b	0.9 × 6.1 (0.9 × 6.1)
Hancock, WI 2008	Prime	pH = 7.0	Plainfield loamy sand (mixed, mesic Typic Udipsamments)	44.11	89.55	4b	1.7 × 1.8 (0.9 × 1.8)
Hancock, WI 2008	Marginal	pH = 4.5	Plainfield loamy sand (mixed, mesic Typic Udipsamments)	44.11	89.55	4b	1.7 × 1.8 (0.9 × 1.8)

† HZ, USDA hardiness zone (http://planthardiness.ars.usda.gov/PHZMWeb/).

‡ Harvested area in parentheses.

Table 2. Switchgrass cultivars included in field evaluation experiments.

Cultivar	Classification group	Ecotype	Geographic origin	Hardiness zones
Alamo	Southern lowland	Lowland	Southern Texas	6, 7, 8, 9
BoMaster	Southern lowland	Lowland	Southeastern USA	6, 7, 8
Performer	Southern lowland	Lowland	Southeastern USA	6, 7, 8
High Tide†	Northern lowland	Lowland	Northeastern Maryland	5, 6, 7
Kanlow	Northern lowland	Lowland	Northern Oklahoma	6, 7, 8
Timber	Northern lowland	Lowland	Southeastern USA	6, 7, 8
Blackwell	Southern upland	Upland	Northern Oklahoma	5, 6, 7
Carthage	Southern upland	Upland	Central North Carolina	5, 6, 7
Cave-in-Rock	Southern upland	Upland	Southern Illinois	4, 5, 6, 7
KY 1625	Southern upland	Upland	Southern West Virginia	5, 6, 7
Pathfinder	Southern upland	Upland	Eastern Nebraska and Kansas	4, 5, 6
Shawnee	Southern upland	Upland	Southern Illinois	4, 5, 6, 7
Summer	Northern upland	Upland	Eastern Nebraska	3, 4, 5, 6
Sunburst	Northern upland	Upland	Eastern South Dakota	3, 4, 5, 6

† High Tide has been classified as upland based solely on phenotype (Cortese et al., 2010). However, it is classified as lowland on the basis of nuclear DNA markers (Cortese et al., 2010) and plastid DNA sequences (Morris et al., 2011), suggesting that it is of hybrid origin.

(all other regions). A sample of biomass (~300–500 g) was weighed, dried at 60°C, and reweighed for dry matter determination. All biomass yields are reported on a dry matter basis. Biomass harvests were conducted for 2 yr with the following exceptions: 1 yr for all four Maryland experiments, and 3 yr for all four Wisconsin experiments and all four Rock Springs, PA, experiments.

Biomass yield data were analyzed using linear mixed models analysis (Littel et al., 1996). Residuals were evaluated for normality and homoscedasticity using quantile-quantile plots and plots of residuals against predicted values (Ghasemi and Zahediasl, 2012). The normal distribution was found to be sufficient for these data, but there was significant variance heterogeneity across regions and across soil categories or N rates within some regions. Mixed models analysis was applied separately to data from each region to evaluate the fixed effects of cultivar, stand age, soil quality category, N rate, and their interactions. Blocks and all interactions involving blocks were assumed to be random effects in these analyses. Mixed models included separate residuals fitted for each combination of soil category and N rate, as necessary according to variance heterogeneity and with the appropriate residual structure chosen using Aikake's information criterion (Littel et al., 1996). The fixed effect of year was treated as a repeated measure with compound symmetry or heterogeneous compound symmetry covariance structures providing the best fit to the data.

Fixed effects of cultivars were evaluated on the basis of both *P*-value and percentage contribution to the sum of squares for all fixed effects involving cultivars. Interactions of cultivars with soil quality category and N rates were evaluated using (i) Kendall's τ as an overall measure of concordance in cultivar ranks across the four environmental conditions and (ii) Spearman's rank correlation as a measure of the rank agreement between prime and marginal soils and between 0- and 100-kg N ha^{-1} fertilizer rates (Conover, 1971).

On the basis of the mixed model ANOVA results, the New Jersey region was split into two subsets, prime versus marginal soils, creating eight geographic regions for all subsequent analyses. For comparative purposes, phenotypic correlations and rank correlations were computed for the cultivar means among these eight regions as a mechanism to evaluate the cultivar × region interaction. Finally, the eight regions were clustered according to the unweighted pair-group method (UPGMA), using the biomass yield of the 14 cultivars as input data.

RESULTS

The main effect of soil quality was significant for six of the seven regions (Table 3). In Wisconsin, where the soil quality difference was due solely to pH, the effect on biomass yield was significant but small (5.97 Mg ha^{-1} for pH 7.0 vs. 5.44 Mg ha^{-1} for pH 4.5; Table 4). In New Jersey, the marginal soil may have been impaired by an infertile A horizon (>10-fold difference in P concentration), causing the largest soil quality main effect in the experiment

Table 3. Linear mixed-model tests of fixed effects for soil quality, nitrogen rate, age of stand, cultivar, and interactions based on 14 switchgrass cultivars evaluated in seven geographical regions.

Fixed effect	df	New Jersey SS†	New Jersey *F*	New Jersey *P*	Rock Springs, PA SS	Rock Springs, PA *F*	Rock Springs, PA *P*	Maryland SS	Maryland *F*	Maryland *P*	Wisconsin SS	Wisconsin *F*	Wisconsin *P*	New York SS	New York *F*	New York *P*	South Dakota SS	South Dakota *F*	South Dakota *P*	Rockton, PA SS	Rockton, PA *F*	Rockton, PA *P*
Soil quality (S)	1		720.06	<0.01		0.79	0.40		22.16	<0.01		6.24	0.02		163.77	<0.01		67.41	<0.01		42.74	<0.01
Nitrogen rate (N)	1		10.40	0.01		17.15	<0.01		1.47	0.23		215.92	<0.01		1.06	0.34		11.11	0.01		273.83	<0.01
N × S	1		0.01	0.93		0.02	0.89		7.22	0.01		0.22	0.64		10.57	0.02		8.50	0.03		75.63	<0.01
Age	1		73.30	<0.01		825.93	<0.01		NA‡	NA		15.72	<0.01		25.41	<0.01		58.54	<0.01		863.98	<0.01
A × S	1		42.42	<0.01		10.20	<0.01		NA	NA		0.34	0.71		22.12	<0.01		148.74	<0.01		20.44	<0.01
A × N	1		0.06	0.81		0.44	0.65		NA	NA		22.62	<0.01		16.69	<0.01		0.17	0.68		0.64	0.42
A × S × N	1		0.14	0.71		8.90	<0.01		NA	NA		0.78	0.47		1.42	0.27		1.31	0.25		13.26	0.00
Cultivar (C)	13	17	1.02	0.43	44	18.51	<0.01	62	2.11	0.02	66	46.44	<0.01	20	4.88	<0.01	52	9.11	<0.01	80	53.52	<0.01
C × S	13	12	0.68	0.78	14	5.77	<0.01	13	0.44	0.95	3	2.13	0.01	6	1.52	0.12	19	4.72	<0.01	6	4.12	<0.01
C × N	13	7	0.54	0.90	12	5.05	<0.01	11	0.36	0.98	10	6.85	<0.01	13	3.30	<0.01	11	1.94	0.03	3	2.03	0.02
C × N × S	13	10	0.59	0.86	7	2.89	<0.01	14	0.47	0.93	2	1.24	0.25	13	3.36	<0.01	7	1.69	0.10	3	2.06	0.02
C × A	13	26	1.54	0.11	16	3.47	<0.01	NA	NA	NA	9	3.23	<0.01	6	1.72	0.06	3	0.67	0.77	3	2.34	0.01
C × A × S	13	15	1.25	0.27	3	0.60	0.94	NA	NA	NA	2	0.67	0.89	28	7.53	<0.01	6	1.76	0.10	3	1.75	0.05
C × A × N	13	8	0.46	0.94	2	0.43	0.99	NA	NA	NA	6	2.25	<0.01	6	1.53	0.11	3	0.55	0.86	1	0.76	0.70
C × A × N × S	13	5	0.38	0.94	2	0.48	0.99	NA	NA	NA	2	0.61	0.94	8	2.20	0.01	<1	0.13	1.00	1	0.73	0.73
		Estimate	*P*		Estimate	*P*		Estimate	*P*		Estimate	*P*		Estimate	*P*		Estimate	*P*		Estimate	*P*	
Rank correlation (N)§		0.23	0.21		0.64	0.01		0.51	0.03		0.88	<0.01		0.46	0.03		0.80	<0.01		0.81	<0.01	
Rank correlation (S)§		−0.08	0.40		0.46	0.05		0.48	0.04		0.85	<0.01		0.20	0.25		0.79	<0.01		0.77	<0.01	
Kendall's τ§		0.29	0.29		0.65	<0.01		0.66	<0.01		0.88	<0.01		0.35	0.11		0.72	<0.01		0.84	<0.01	

† SS, percentage of the sum of squares for all eight main effects and interactions involving cultivars.

‡ NA, not applicable due to a single year of data for all trials in Maryland.

§ Rank correlation (N) is the pooled rank correlation coefficient between the two nitrogen rates; Rank correlation (S) is the pooled rank correlation between the two soil categories; Kendall's τ is the coefficient of concordance measuring the agreement in ranking across all four experiments within each region.

Table 4. Mean biomass yield for four field experiments of switchgrass conducted under differing soil quality and nitrogen (N) rates within seven geographical regions.

Soil quality and N rate	New Jersey		Rock Springs, PA		Maryland		Wisconsin		New York		South Dakota		Rockton, PA	
	Mg ha^{-1}													
Prime, 100 kg N ha^{-1}	11.88	a†	9.47	a	4.34	a	7.60	a	1.65	c	6.23	a	13.20	a
Prime, 0 kg N ha^{-1}	10.84	a	7.70	b	2.91	b	4.34	b	2.48	c	4.48	b	9.01	c
Marginal, 100 kg N ha^{-1}	3.08	b	9.06	a	5.07	a	6.97	a	7.24	a	3.71	b	12.85	a
Marginal, 0 kg N ha^{-1}	2.02	b	7.39	b	5.61	a	3.91	b	5.63	b	3.59	b	11.54	b

† Means followed by different letters are significantly different based on pairwise LSD (P =0.05). These were preplanned comparisons.

(11.36 vs. 2.55 Mg ha^{-1}, Table 4). Results for the other five sites were mixed, with biomass yields not always favored by the "prime" site. In two cases, Maryland and New York, this was due to severe weed problems during establishment on the prime site, resulting in an impact on biomass yield that lasted throughout the duration of the study (data not shown).

Likewise, the impact of N fertilizer was highly variable across the seven regions (Tables 3 and 4). Application of N fertilizer increased biomass yield by 18 to 63% at New Jersey, South Dakota, and Wisconsin ($P < 0.01$), but only 5 to 9% at the two Pennsylvania regions ($P < 0.01$). Conversely, there was no effect of N fertilizer in New York or Maryland. Across the seven regions, the prime (100-kg N ha^{-1}) treatment ranked highest in biomass yield of the four soil quality–fertilization treatments, with only Maryland and New York as exceptions (Table 4). Again, this was likely due to weed competition in prime plots at these two locations.

Cultivar means were significantly different in six of the seven regions, accounting for 44 to 80% of the variation associated with cultivars or cultivar × environment interactions, excluding New Jersey and New York (Table 3). Averaged across all environmental factors, cultivar means were not significantly different in New Jersey due to the strong GE interactions. For these six regions, cultivar × environment interactions were small compared with the cultivar main effect. The cultivars were selected to represent a wide geographic region, largely because the study itself represented a broad region, from USDA Hardiness Zone 4 to 7 and from 75 to 100° longitude, but also because little is known about the GE interactions of switchgrass cultivars associated with soil characteristics. As such, much of the genetic variation in this study was associated with cultivar groups, described largely by ecotype and region of origin (Table 5), and this was also true for New Jersey (Table 6). For New York, the cultivar × environment interactions were stronger than for most sites, but there was still significant cultivar variation averaged across soils and N rates and a moderate level of concordance in cultivar rankings (Table 3).

Three fairly clear patterns emerged from the data in Tables 5 and 6. First, lowland cultivars tended to have higher biomass yield in New Jersey and both Pennsylvania regions. These represented three of the four regions in USDA Hardiness Zones 6 and 7, with Maryland being the only exception. Conversely, upland cultivars tended to have higher biomass yield in Wisconsin, New York, and South Dakota, representing Hardiness Zones 4 and 5. Second, northern-origin lowland cultivars had higher biomass yield than southern-origin lowland cultivars within all seven regions (Table 5). Differences between northern-origin and southern-origin upland cultivars were unstable and inconsistent, not necessarily associated with specific characteristics of the seven regions. Lastly, the pattern of variation within the New Jersey region could only be observed by examining group means separately for each of the four soil quality–N treatments due to strong GE interactions, as evidenced by the low rank correlations

Table 6. Mean biomass yield of four groups of switchgrass cultivars evaluated under four soil qualities and nitrogen levels in New Jersey.

Cultivar group	n	Prime soil 100 kg N ha^{-1}		Prime soil 0 kg N ha^{-1}		Marginal soil 100 kg N ha^{-1}		Marginal soil 0 kg N ha^{-1}	
		Mg ha^{-1}							
Southern lowland	3	12.37	a†	10.23	b	2.88	a	1.56	c
Northern lowland	3	12.01	a	12.11	a	3.22	a	1.94	bc
Southern upland	6	12.13	a	10.85	bc	3.07	a	2.26	a
Northern upland	2	10.19	b	9.81	c	3.20	a	2.10	ab

† Means followed by different letters are significantly different based on pairwise LSD (P =0.05). These were preplanned comparisons.

Table 5. Mean biomass yield of four groups of switchgrass cultivars evaluated in seven regions.

Cultivar group	n	New Jersey		Rock Springs, PA		Maryland		Wisconsin		New York		South Dakota		Rockton, PA	
		Mg ha^{-1}													
Southern lowland	3	6.76	b†	8.24	c	3.42	c	2.92	d	3.74	c	1.18	d	12.40	b
Northern lowland	3	7.32	a	8.84	a	4.83	ab	4.61	c	4.08	b	3.26	c	13.58	a
Southern upland	6	7.08	ab	8.68	b	4.58	b	7.08	b	4.50	a	6.29	a	11.25	c
Northern upland	2	6.32	c	7.16	d	5.28	a	7.42	a	4.54	a	5.99	b	8.85	d

† Means followed by different letters are significantly different based on pairwise LSD (P =0.05). These were preplanned comparisons.

between soil types and N rates (Tables 3 and 6). The superiority of the lowland cultivars compared with the upland cultivars was most obvious for the prime soil, and the difference between northern-origin and southern-origin lowland cultivars was observed only for the prime soil without N fertilizer. Group mean differences were very similar for the New Jersey prime location compared with the two Pennsylvania regions, while the New Jersey marginal location was something of an oddity due to low mean yields and relatively small differences.

There were strong GE interactions present across the geographic breadth of this study, as evidenced by phenotypic and rank correlations between regions (Table 7). Only 5 of 28 phenotypic correlations and 5 of 28 rank correlations were significant. The significant correlations pointed out two distinct regional groups, which can be seen in the cluster dendrogram (Fig. 1). One group consists of the two Pennsylvania regions combined with the New Jersey prime site. These three regions had phenotypic and rank correlations >0.70 (Table 7) and were the locations in which lowland cultivars had the greatest biomass yield advantage. The other group was a loose association of the other five regions in which upland cultivars had the biomass yield advantage or there were no differences among cultivar groups. South Dakota and Wisconsin, the two regions representing Hardiness Zone 4, formed the strongest association within this group, as expected according to results from Table 5. New York and Maryland had consistently positive correlations with these two regions, but there were not sufficient degrees of freedom for these correlations to be significant.

The general lack of GE interactions associated with the two environmental effects, soil quality and N fertilizer, are illustrated in Fig. 2. For all regions except New Jersey and New York, there was a fairly strong and consistent relationship between the two N rates (left side of Fig. 2) and between the two soil quality levels (right side of Fig. 2). For these five regions, most of the rank changes between the two N rates or the two soil qualities were fairly minor. With the exception of New Jersey, rank correlation coefficients ranged from 0.51 to 0.88 between the two N rates and from 0.47 to 0.85 between the two soil

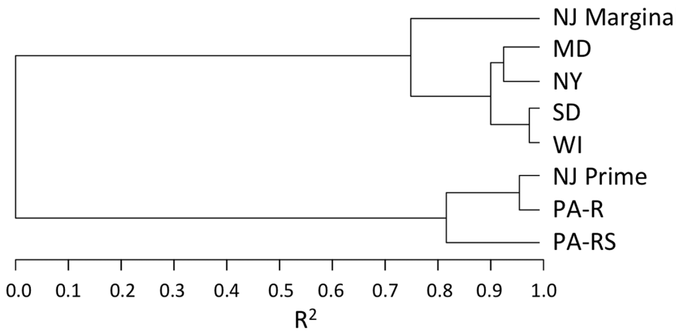

Fig. 1. Cluster dendrogram showing linkages between eight switchgrass evaluation regions based on the mean biomass yield of 14 cultivars evaluated in four experiments per region (or two experiments within each of the two New Jersey regions). PA-R, Rockton, PA; PA-RS, Rock Springs, PA.

qualities (Table 3). Kendall's τ, a measure of concordance of cultivar rankings across the four environmental treatments, ranged from 0.66 to 0.88. New Jersey was the only exception, with low rank correlations, low concordance, and clearly little relationship between N rates or soil qualities (Table 3, Fig. 2).

Lastly, the GE interaction on a broad scale, across regions, can be placed in context using mean cultivar rankings within each region, again treating the New Jersey prime and marginal sites as two distinct regions (Table 8). Seven of the fourteen switchgrass cultivars in the experiment ranked as the top cultivar for at least one of these eight regions, illustrating the very strong GE interaction across the breadth of these regions. Even within the two groups of fairly similar regions, as shown in the cluster dendrogram (Fig. 1), different regions ranked a different cultivar as the top cultivar (e.g., 'Cave-in-Rock' in Wisconsin, 'Carthage' in South Dakota, 'Blackwell' in Maryland, and 'Kanlow' and 'KY 1625' in New York). The same was true in the other cluster group, with 'Timber', Kanlow, and Carthage ranking highest at these three sites.

DISCUSSION

Prime versus Marginal Growing Conditions

The inconsistent differences between prime and marginal sites on the basis of overall means indicated that predictions of "good" versus "bad" sites to grow switchgrass for

Table 7. Phenotypic correlation coefficients (above the diagonal) and rank correlation coefficients (below the diagonal) for mean biomass yield estimated in seven geographical regions, with one region (New Jersey) split into prime soil and marginal soil.†

	New Jersey prime	New Jersey marginal	Rock Springs, PA	Maryland	Wisconsin	New York	South Dakota	Rockton, PA
New Jersey prime		0.07	0.82	−0.28	−0.22	0.12	0.03	0.71
New Jersey marginal	0.13		−0.01	0.23	0.34	−0.23	0.14	−0.21
Rock Springs, PA	0.75	0.02		−0.30	−0.13	0.40	0.14	0.83
Maryland	−0.20	0.05	−0.18		0.45	0.07	0.42	−0.21
Wisconsin	−0.27	0.30	−0.16	0.44		0.43	0.75	−0.52
New York	0.17	−0.04	0.43	0.11	0.39		0.69	0.69
South Dakota	0.08	0.12	0.21	0.34	0.56	0.71		−0.17
Rockton, PA	0.71	−0.19	0.84	−0.12	−0.53	0.16	−0.05	

† Critical values for significance are 0.53 (P = 0.05) and 0.66 (P = 0.01).

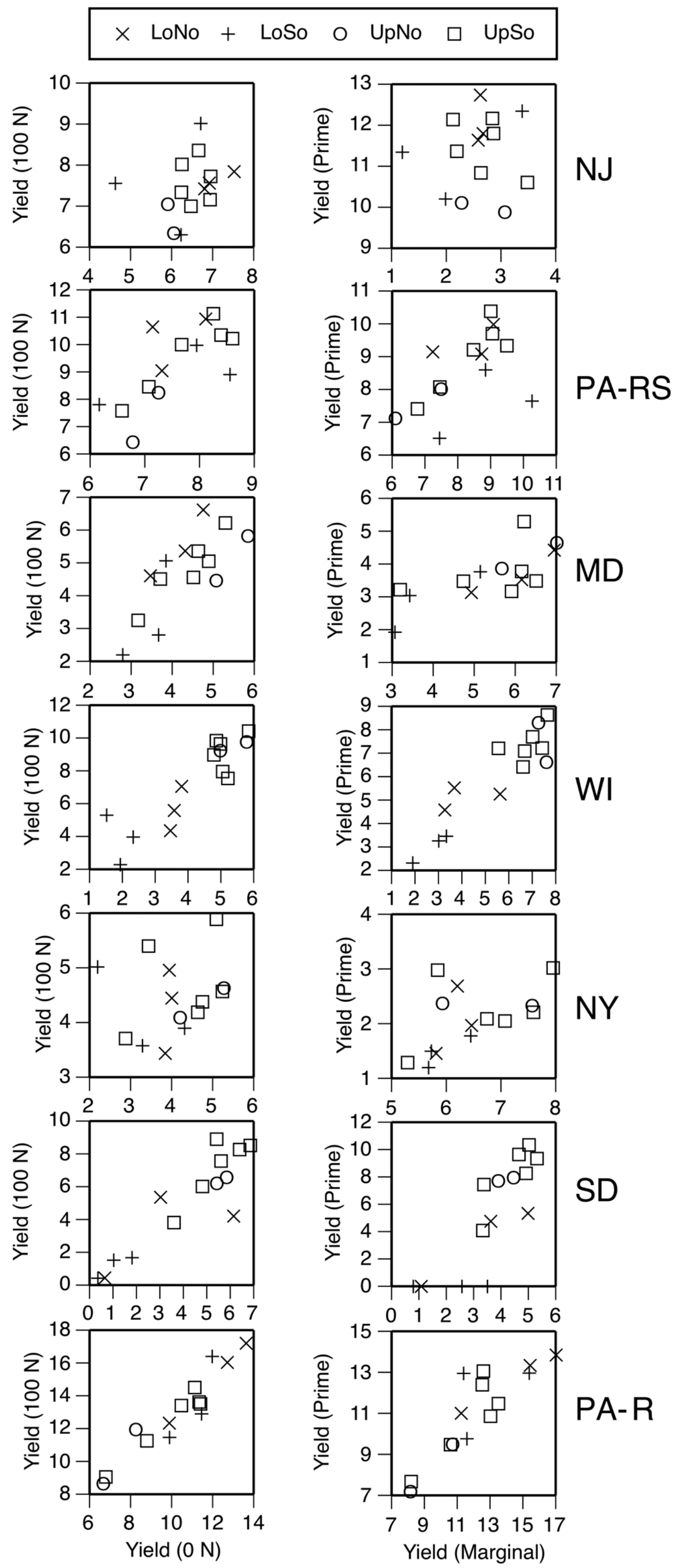

Fig. 2. Scatterplots of 14 switchgrass cultivars, showing the relationships of mean biomass yield for nitrogen-fertilized plots versus unfertilized plots (left side) and plots on prime versus marginal soil (right side) for seven evaluation regions (top to bottom: NJ, New Jersey; PA-RS, Rock Springs, PA; MD, Maryland; WI, Wisconsin; NY, New York; SD, South Dakota; and PA-R, Rockton, PA). Note that scale varies across the 14 panels.

biomass are not always accurate. The study was focused on creating clear and obvious differences in soil characteristics with minimal confounding of climatic factors between pairs of locations within a region. Confounding factors, such as differential weediness observed in New York and Maryland, may be impossible to predict and control. While the experiment at Hancock was ideal, it is rare to find such a clear and distinct design arrangement that isolates a single soil factor, such as pH, which is possible only after many years of repeated soil amendments. Despite this shortcoming, some conclusions are possible from this study.

Nitrogen fertilization studies conducted in the Great Plains area of the United States suggest that ~10 kg N ha^{-1} is required for each additional Mg ha^{-1} of dry biomass yield (Casler et al., 2012). Parrish and Fike (2005) describe the issue of N requirements for biomass yield as "unsettled." A meta-analysis of numerous published reports supports Parrish and Fike's conclusions, especially for the lowland ecotype (Wullschleger et al., 2010). As observed in the current study, high rates of N fertilization do not guarantee high biomass yields, or a significant response to N fertilizer. In the current study, biomass yield under the high N rate ranged from 10 to 76% higher than in sites without nitrogen fertilizer, with nine positive responses and five neutral responses (Table 4). Hong et al. (2014) found significant responses to N fertilization in only 6 of 19 location-years and one negative response, interestingly at the same location where we observed a negative response (Ithaca, NY). The average N response of this study was 1.54 Mg ha^{-1}, a 26% increase, while the maximum was 4.19 Mg ha^{-1}, a 78% increase, both far lower than the generalized response suggested by Casler et al. (2012). The largest responses occurred at the sites with the highest biomass yields, and many of our sites had extremely low yields, generally at the low end of values in the meta-analysis of Wullschleger et al. (2010). For many of these sites, it is likely that soil N was not a factor limiting biomass yield.

Of the 14 soil quality sites within the seven regions, only five (the New Jersey prime site and all four Pennsylvania sites) had mean biomass yields that would rank moderate to high against published literature (Wullschleger et al., 2010) or be considered economically sustainable when compared with the production costs of Perrin et al. (2008). This is the first report that growing conditions in New Jersey can produce competitive switchrass yields compared with other regions of the country. Clearly there were other factors limiting biomass yield at many of these sites, and some of these factors likely played a role in limiting N responses as well. While it is tempting to blame high soil N as a factor that tends to cause reduced or null responses to N fertilization, the extremely low biomass yields at many of these sites (Table 4) suggest that there are probably other environmental factors limiting biomass yield.

Table 8. Mean rank value for biomass yield of 14 switchgrass cultivars evaluated in four experiments within each of eight regions (lowest possible value = 1, corresponding to the highest mean yield, and highest possible value = 14, corresponding to the lowest mean yield).

Cultivar†	New Jersey prime‡	New Jersey marginal‡	Rock Springs, PA	Maryland	Wisconsin	New York	South Dakota	Rockton, PA
Alamo	8	13	6	14	14	8	11	3
Blackwell	9	10	5	5	6	2	5	6
BoMaster	4	7	6	11	12	9	10	6
Carthage	5	6	3	8	7	5	2	6
Cave-in-Rock	6	5	4	12	1	7	8	8
High Tide	7	9	8	10	10	11	12	9
Kanlow	7	7	8	3	11	8	6	1
KY 1625	11	3	10	3	4	12	10	11
Pathfinder	11	6	13	9	6	7	2	14
Performer	10	12	12	8	13	12	13	9
Shawnee	5	11	5	6	5	6	4	6
Summer	14	4	9	7	5	5	6	11
Sunburst	13	9	13	3	3	8	5	14
Timber	2	6	4	6	9	6	9	3

† Highest-ranked cultivar within each column, including ties. Rankings were computed from cultivar means within four field trials (or two for the two New Jersey sites), then mean ranks were computed across the field trials within the eight regions above.

‡ Ranks were averaged across only two field trials for New Jersey prime and marginal sites.

Our ability to create differential environmental conditions on the basis of soil quality was also characterized by inconsistent responses. There was a small and significant response to pH in Wisconsin and a large response to differential P fertility of the A horizon in New Jersey. Soil quality effects within the remaining five regions were based on an assumption that soil drainage would have an impact on biomass yields. For those sites with differential depth of AB horizons, we expected the shallower AB horizons to result in lower yields, more stress, and perhaps differential cultivar rankings. There is precedent that reduced depth of AB horizons overlying a claypan results in reduced switchgrass biomass yields (Yost et al., 2017). Across the regions of our study, however, this effect was highly variable and unpredictable. Switchgrass is a highly resilient species, capable of a considerable amount of phenotypic plasticity in response to environmental stimuli (Casler et al., 2004, 2007). In all likelihood, New Jersey was the only region in which significant additional stress was placed on the switchgrass plants due to soil quality. For the remaining six regions, the impact of the soil quality factor on biomass yield of switchgrass, while often significant and repeatable, was not sufficient to drastically alter either the mean yield or the cultivar rankings, with the sole exception of New York. This was also observed in the study of Casler et al. (2007)—across numerous field sites, the effects of temperature, photoperiod, and precipitation were so large that soil quality effects could not be detected. Unfortunately, the cultivar × soil quality interaction in New York could not be attributed to a single factor due to severe confounding between drainage and weediness. The extremely low biomass yields of the prime New York site suggest that weediness may have been the dominant environmental factor between the prime and marginal sites in New York, possibly causing the GE interaction observed at this location (Table 3, Fig. 2).

Implications for Switchgrass Breeding and Evaluation

Breeding and evaluating new cultivars of switchgrass is heavily complicated by GE interactions. Temperature and photoperiod are the two most dominant environmental factors that regulate GE interactions, such that usual cultivar recommendations involve deployment of a cultivar no more than one hardiness zone from its site of origin (Casler et al., 2007; Casler, 2012). Precipitation also has an impact on adaptation and ranking of cultivars: cultivars of eastern origin are not adapted to extreme dryland conditions, and cultivars of western origin tend to succumb to severe disease problems in the more humid eastern United States (Berdahl et al., 2005; Casler et al., 2007; Casler, 2012). On the basis of these studies, one proposal has suggested a need for at least eight regional breeding pools of switchgrass germplasm to optimize biomass yields within each region (Casler, 2012; Casler et al., 2015). Two of these breeding pools would be located in the north-central and northeastern United States.

Our study supports this proposal, specifically in the need for distinct breeding populations and cultivars in the northcentral versus northeastern United States. The three most productive sites in our study—the prime New Jersey site and the four Pennsylvania sites—had highly unique cultivar rankings, especially compared with those observed in South Dakota and Wisconsin. These five sites clearly favored lowland cultivars over upland cultivars, while the South Dakota and Wisconsin sites showed the opposite response, as they have done in numerous previous studies (Casler, 2012; Casler et al., 2015). Results from the marginal New Jersey site, Maryland, and New York were more equivocal, but that may have simply been due to the low biomass yields at these sites, compressing variability among cultivars and limiting the ability of some cultivars to express their phenotype. Currently, there are three breeding programs in the northeastern United States or eastern Canada (New Jersey, New York, and Québec). Each of these programs is based on the use of both upland and lowland germplasm, largely as independent populations undergoing selection for high biomass yield and adaptation to local conditions. Because both upland and lowland cultivars performed well in the eastern regions of this study, there is clearly merit in continuing to breed both ecotypes within the eastern United States and Canada.

More problematic and more difficult to resolve, though, is the question of soil quality and specifically how switchgrass breeders should develop cultivars for use on marginal sites. Through the use of paired sites within regions, we successfully eliminated pH as a factor potentially affecting switchgrass cultivar rankings. While we have not eliminated soil drainage as a potential factor, it was clear from this study that it was not an important factor at the level it was represented. Switchgrass possesses genetic variability for drought tolerance, which is expressed as cultivar variation in response to drought (Byrd and May, 2000; Aspinwall et al., 2013). This variability is probably partly responsible for the GE interactions that are manifested as east–west adaptive limitations, especially between the more arid Great Plains and the more humid eastern United States (Casler et al., 2007). There is also some evidence for genetic variation in flooding tolerance, with the lowland ecotype showing significantly better adaptation to flooded soils (Porter, 1966). We found no evidence or corroboration for that in the current study, probably because the proper environmental conditions were not sufficiently expressed.

In contrast, the difference in depth and fertility of the A horizon of the two New Jersey sites had a significant influence on both mean biomass yield and cultivar rankings. The impact of this factor was so great as to make the New Jersey prime site appear most similar to the two high-yielding Pennsylvania sites and the New Jersey marginal site appear most similar to the other sites with relatively low biomass yields (Fig. 1). This result is similar to recent results of Brown et al. (2016), who showed that topsoil replacement was essential to achieve high biomass yields on reclaimed mines. While switchgrass is inherently a P-thrifty species, fertilization with P can result in increased biomass yields, indicating that P can be limiting at some sites (Parrish and Fike, 2005). We suspect that the GE interactions observed in New Jersey may be due to a combination of both differential P and differential depth of the A horizon. Furthermore, there was a strong interaction between cultivars and reclaimed mine sites that differed in presence or absence of topsoil, with a strong negative rank correlation between sites (Marra et al., 2013), results that were remarkably similar to those of the New Jersey sites.

Finally, this is the first study to comprehensively examine the specific interaction of switchgrass cultivars with N fertilization rates. This interaction dramatically affected the relative performance of the four cultivar groups in New Jersey, but not within the other six regions. This result implies that N fertilization does not, in general, affect switchgrass cultivar rankings and, by extension, should not affect breeding objectives (i.e., new cultivars bred under conditions of N fertilization should still be superior when no N fertilizer is applied). However, such a conclusion ignores several fundamental lines of reasoning that point to the opposite conclusion.

First, if Sewell Wright's theory of shifting balance is correct (Wright, 1982), breeders must recognize this before creating breeding objectives and setting long-term goals. The shifting balance theory supports the age-old breeder's axiom, "you get what you select for," as in there are multiple fitness peaks and multiple selection landscapes, and maximizing breeding goals requires breeders to choose the proper landscape for the desired goal. Rose et al. (2007) reported a rank correlation coefficient of only 0.14 for 40 switchgrass genotypes evaluated in HYEs versus LYEs. Furthermore, they showed higher rates of gain from selection for high biomass yield in the LYE compared with the HYE. Nitrogen fertilization was one of the principal factors differing between HYEs and LYEs (90 vs. 0 kg N ha^{-1}).

Second, application of N fertilizer to switchgrass breeding nurseries completely eliminates the possibility of discovering and taking advantage of microbial associations that would allow switchgrass to use atmospheric N in its growth cycle (Veresoglou et al., 2011). Associations of perennial C4 grasses with N-fixing microbes have been known for many years (Brejda et al., 1994; Boddey et al., 2003; Miyamoto et al., 2004) and may be a mechanism to develop switchgrass cultivars that are better able to scavenge N and other nutrients from the soil.

Third, perennial grasses such as switchgrass recycle much of their tissue N into root and crown tissues for the winter dormancy period (Lemus et al., 2008; Schwartz and Amasino, 2013). There is considerable genetic variation for N concentration in the biomass and for recycling efficiencies (Yang et al., 2009). There is also genetic variation within switchgrass for N requirement; Porter (1966) showed that severe reductions in available N reduced biomass of the lowland ecotype by only 42%, but reduced biomass of the upland ecotype by 73%. Repeated applications of N fertilizer to breeding nurseries would eliminate the opportunity to select genotypes with better N-scavenging ability and lower N requirements for normal growth.

Fourth, N fertilizer is the single most expensive input in a switchgrass biomass production system (Perrin et al., 2008). Nitrogen fertilization leads to higher concentrations of N in the biomass, which is removed on harvest (Lemus et al., 2008; Jung and Lal, 2011) and is wasted, even detrimental, once it enters any type of thermochemical conversion pipeline (McKendry, 2002; Boateng et al., 2006). Nitrogen fertilization also leads to increased N_2O emissions, a harmful greenhouse gas (Erisman et al., 2010). Furthermore, N fertilization is an inefficient process, with increasing rates of N resulting in reduced N-use efficiency and N recovery (Lemus et al., 2008; Owens et al., 2013).

CONCLUSIONS

If the development of switchgrass as a sustainable biomass feedstock depends on its extensive use on marginal lands, switchgrass breeders should find a mechanism to conduct their breeding and evaluation research on qualified marginal lands. As Richards et al. (2014) pointed out, marginal lands can be marginal for a number of reasons, sometimes multiple reasons, as is the case with the manipulated "marginal" environment (the LYE) of Rose et al. (2007). Breeding resources (i.e., time and funding) are too scarce to define multiple types of marginal lands and conduct independent breeding programs under different environmental conditions. Rather, breeders must be content to define a type of environment that is accessible, repeatable, affordable, and workable within time and budgetary constraints. As in the case of Rose et al. (2007), this may involve reducing or eliminating a number of inputs and resources, such as irrigation, fertilizer, herbicides, etc.

Breeding switchgrass under high-input or optimal conditions for commercial production of switchgrass under low-input or marginal conditions is a form of indirect selection (i.e., selection of one trait to obtain a positive correlated response in another trait). In practice, indirect selection often fails because the genetic correlation between the selected trait and the response trait are small or nonexistent, as in the case of Rose et al. (2007) with HYEs and LYEs. Brummer and Casler (2014) provide a number of examples illustrating this principle. A further disadvantage of indirect selection would arise under shifting balance, which could easily prevent a long-term, indirect-selection program from achieving its ultimate goal (Wright, 1982).

There are two potentially complicating factors to drawing a final conclusion that all switchgrass breeding for high-biomass cultivars should be conducted under low-input conditions that do not include N fertilizer applications. First, switchgrass is currently under production to generate biomass for combustion in the eastern United States and Canada. Many of these production regions overlap with livestock production areas, creating the possibility of using manure to fertilize switchgrass intended for biomass production (Lee et al., 2007). Frequent or repeated use of manure on switchgrass could elevate production fields to "prime" status by increasing soil fertility and organic matter. If, as suggested by Rose et al. (2007), switchgrass selected and developed in low-input environments is also superior in high-input environments, all the better. However, a shifting balance scenario could have the same impact as described above if the long-term selection process concentrates only on those alleles that allow the plants to perform well in low-input environments.

Second, the extensive use of switchgrass as a biomass crop in buffer strips along surface waters could also create "prime" production environments without consciously using high-input approaches. Most surface waters are located at the bottom of valleys, glens, or dales that channel runoff to the buffer strip. Runoff would contain sediments and nutrients that would benefit the switchgrass crop, potentially resulting in similar effects as manure-treated switchgrass described above.

In the end, these decisions will lie with each individual breeder, being dependent on many factors including funding, land and labor restrictions, accessibility of field sites, perceptions of local production needs and strategies, and politics. The last factor overarches nearly every decision a breeder would make, from the initial decisions about setting up the breeding program (funding availability and restrictions) all the way to the final production scenario for the new cultivar (government support and policies regarding biomass crop production).

Conflict of Interest

The authors declare there to be no conflict of interest.

Acknowledgments

The authors would like to thank Donald Viands and his breeding crew at Cornell University for support in data collection for the New York location. Funding for this project was provided by USDA-NIFA Grant No. 2009-10001-05116, by AFRI Competitive Grant No. 2012-68005-19703 from the USDA National Institute of Food and Agriculture, and the New Jersey Agricultural Experiment Station.

References

Aspinwall, M.J., D.B. Lowry, S.H. Taylor, T.E. Juenger, C.V. Hawkes, M.V. Johnson et al. 2013. Genotypic variation in traits linked to climate and aboveground productivity in a widespread C4 grass: Evidence for a functional trait syndrome. New Phytol. 199:966–980. doi:10.1111/nph.12341

Berdahl, J.D., A.B. Frank, J.M. Krupinsky, P.M. Carr, J.D. Hanson, and H.A. Johnson. 2005. Biomass yield, phenology, and survival of diverse switchgrass cultivars and experimental strains in western North Dakota. Agron. J. 47:1082–1090.

Boateng, A.A., K.B. Hicks, and K.P. Vogel. 2006. Pyrolysis of switchgrass (*Panicum virgatum*) harvested at several stages of maturity. J. Anal. Appl. Pyrolysis 75:55–64. doi:10.1016/j.jaap.2005.03.005

Boddey, R.M., S. Urquiaga, B.J.R. Alves, and V. Reis. 2003. Endophytic nitrogen fixation in sugarcane: Present knowledge and future applications. Plant Soil 252:139–149. doi:10.1023/A:1024152126541

Brejda, J.J., R.J. Kremer, and J.R. Brown. 1994. Indications of associative nitrogen fixation in Eastern gamagrass. J. Range Manage. 47:192–195. doi:10.2307/4003014

Brown, C., T. Griggs, T. Keene, M. Marra, and J. Skousen. 2016. Switchgrass biofuel production on reclaimed surface mines: I. Soil quality and dry matter yield. BioEnergy Res. 9:23–31.

Brummer, E.C., W.T. Barber, S.M. Collier, T.S. Cox, R. Johnson, S.C. Murray et al. 2011. Plant breeding for harmony between agriculture and the environment. Front. Ecol. Environ 9:561–568. doi:10.1890/100225

Brummer, E.C., and M.D. Casler. 2014. Yield gains in cool-season forage legumes, cool-season forage grasses, and switchgrass. In: S. Smith, B. Diers, J. Specht, and B. Carver, editors, Genetic gain in major U.S. field crops. ASA, CSSA, SSSA, Madison, WI. p. 33–51.

Byrd, G.T., and P.A. May, II. 2000. Physiological comparisons of switchgrass cultivars differing in transpiration efficiency. Crop Sci. 40:1271–1277. doi:10.2135/cropsci2000.4051271x

Casler, M.D. 2012. Switchgrass breeding, genetics, and genomics. In: A. Monti, editor, Switchgrass. Springer, New York. p. 29–53. doi:10.1007/978-1-4471-2903-5_2

Casler, M.D. 2014. Heterosis and reciprocal-cross effects in tetraploid switchgrass. Crop Sci. 54:2063–2069. doi:10.2135/cropsci2013.12.0821

Casler, M.D., R.B. Mitchell, and K.P. Vogel. 2012. Switchgrass. In: C. Kole, C.P. Joshi, and D.R. Shonnard, editors, Handbook of bioenergy crop plants. Vol. 2. Taylor & Francis, New York. p. 563–590. doi:10.1201/b11711-25

Casler, M.D., and K.P. Vogel. 2014. Selection for biomass yield in upland, lowland, and hybrid switchgrass. Crop Sci. 54:626–636. doi:10.2135/cropsci2013.04.0239

Casler, M.D., K.P. Vogel, and M. Harrison. 2015. Switchgrass germplasm resources. Crop Sci. 55:2463–2478. doi:10.2135/cropsci2015.02.0076

Casler, M.D., K.P. Vogel, C.M. Taliaferro, N.J. Ehlke, J.D. Berdahl, E.C. Brummer et al. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Sci. 47:2249–2260. doi:10.2135/cropsci2006.12.0780

Casler, M.D., K.P. Vogel, C.M. Taliferro, and R.L. Wynia. 2004. Latitudinal adaptation of switchgrass populations. Crop Sci. 44:293–303. doi:10.2135/cropsci2004.2930

Conover, W.J. 1971. Practical nonparametric statistics. John Wiley & Sons, New York.

Cortese, L.M., J. Honig, C. Miller, and S.A. Bonos. 2010. Genetic diversity of twelve switchgrass populations using molecular and morphological markers. BioEnergy Res. 3:262–271. doi:10.1007/s12155-010-9078-2

Erisman, J.W., H. van Grinsven, A. Leip, A. Mosier, and A. Bleeker. 2010. Nitrogen and biofuels; an overview of the current state of knowledge. Nutr. Cycling Agroecosyst. 86:211–223. doi:10.1007/s10705-009-9285-4

Gelfand, I., R. Sahajpal, X. Zhang, R.C. Izaurralde, K.L. Gross, and G.P. Robertson. 2013. Sustainable bioenergy production from marginal lands in the US Midwest. Nature. doi:10.1038/nature11811

Ghasemi, A., and S. Zahediasl. 2012. Normality tests for statistical analysis: A guide for non-statisticians. Int. J. Endocrinol. Metab. 10:486–489. doi:10.5812/ijem.3505

Gopalakrishnan, G., M.C. Negri, and S.W. Snyder. 2011. A novel framework to classify marginal land for sustainable biomass feedstock production. J. Environ. Qual. 40:1593–1600. doi:10.2134/jeq2010.0539

Hernandez-Santana, V., X. Zhou, M.J. Helmers, H. Asbjornsen, R. Kolka, and M. Tomer. 2013. Native prairie filter strips reduce runoff from hillslopes under annual row-crop systems in Iowa, USA. J. Hydrol. 477:94–103. doi:10.1016/j.jhydrol.2012.11.013

Hong, C.O., V.N. Owens, D. Bransby, R. Farris, J. Fike, E. Heaton et al. 2014. Switchgrass response to nitrogen fertilizer across diverse environments in the USA: A regional feedstock partnership report. BioEnergy Res. 7:777–788. doi:10.1007/s12155-014-9484-y

Jung, J.Y., and R. Lal. 2011. Impacts of nitrogen fertilization on biomass production of switchgrass (*Panicum virgatum* L.) and changes in soil organic carbon in Ohio. Geoderma 166:145–152. doi:10.1016/j.geoderma.2011.07.023

Kang, S., W.M. Post, J.A. Nichols, D. Wang, T.O. West, V. Bandaru, and R.C. Izaurralde. 2013. Marginal lands: Concept, assessment, and management. J. Agric. Sci. 5:129–139. doi:10.5539/jas.v5n5p129

Lee, D.K., V.N. Owens, and J.J. Doolittle. 2007. Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on Conservation Reserve Program land. Agron. J. 99:462–468. doi:10.2134/agronj2006.0152

Lemus, R., D.J. Parrish, and O. Abaye. 2008. Nitrogen-use dynamics in switchgrass grown for biomass. BioEnergy Res. 1:153–162. doi:10.1007/s12155-008-9014-x

Littel, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.

Marra, M., T. Keene, J. Skousen, and T. Griggs. 2013. Switchgrass yield on reclaimed surface mines for bioenergy production. J. Environ. Quality 42:696–703. doi:10.2134/jeq2012.0453

McKendry, P. 2002. Energy production from biomass (part 3): Gasification technologies. Bioresour. Technol. 83:55–63. doi:10.1016/S0960-8524(01)00120-1

McLaughlin, S.B., and L.A. Kzsos. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass Bioenergy 28:515–535. doi:10.1016/j.biombioe.2004.05.006

Milbrandt, A.R., D.M. Heimiller, A.D. Perry, and C.B. Field. 2014. Renewable energy potential on marginal lands in the United States. Renew. Sustain. Energy Rev. 29:473–481. doi:10.1016/j.rser.2013.08.079

Mitchell, R.B., K.P. Vogel, and D.R. Uden. 2012. The feasibility of switchgrass for biofuel production. Biofuels 3:47–59. doi:10.4155/bfs.11.153

Miyamoto, T., M. Kawahara, and K. Minamisawa. 2004. Novel endophytic nitrogen-fixing Clostridia from the grass *Miscanthus sinensis* as revealed by terminal restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 70:6580–6586. doi:10.1128/AEM.70.11.6580-6586.2004

Morris, G.P., P.P. Grabowski, and J.O. Borevitz. 2011. Genomic diversity in switchgrass (*Panicum virgatum*): From the continental scale to a dune landscape. Mol. Ecol. 20:4938–4952. doi:10.1111/j.1365-294X.2011.05335.x

Owens, V.N., D.R. Viands, H.S. Mayton, J.H. Fike, R. Farris, E. Heaton et al. 2013. Nitrogen use in switchgrass grown for bioenergy across the USA. Biomass Bioenergy 58:286–293. doi:10.1016/j.biombioe.2013.07.016

Parrish, D.J., and J.H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. 24:423–459. doi:10.1080/07352680500316433

Perrin, R., K.P. Vogel, M.R. Schmer, and R.B. Mitchell. 2008. Farm-scale production cost of switchgrass for biomass. BioEnergy Res. 1:91–97. doi:10.1007/s12155-008-9005-y

Porter, C.T. 1966. An analysis of variation between upland and lowland switchgrass, *Panicum virgatum* L., in central Oklahoma. Ecology 47:980–992. doi:10.2307/1935646

Porter, P.A., R.B. Mitchell, and K.J. Moore. 2015. Reducing hypoxia in the Gulf of Mexico: Reimagining a more resilient agricultural landscape in the Mississippi River Watershed. J. Soil Water Conserv. 70:63A–68A. doi:10.2489/jswc.70.3.63A

Richards, B.K., C.R. Stoof, I.J. Cary, and P.B. Woodbury. 2014. Reporting on marginal lands for bioenergy feedstock production: A modest proposal. BioEnergy Res. 7:1060–1062. doi:10.1007/s12155-014-9408-x

Rose, L.W., IV, M.K. Das, R.G. Fuentes, and C.M. Taliaferro. 2007. Effects of high- vs. low-yield environments on selection for increased biomass yield in switchgrass. Euphytica 156:407–415. doi:10.1007/s10681-007-9390-x

Sanderson, M.A., P.R. Adler, A.A. Boateng, M.D. Casler, and G. Sarath. 2006. Switchgrass as a biofuels feedstock in the USA. Can. J. Plant Sci. 86:1315–1325. doi:10.4141/P06-136

Schwartz, C., and R. Amasino. 2013. Nitrogen recycling and flowering time in perennial bioenergy crops. Front. Plant Sci. doi:10.3389/fpls.2013.00076

Shen, Z., D.J. Parrish, D.D. Wolf, and G.E. Welbaum. 2001. Stratification in switchgrass seeds is reversed and hastened by drying. Crop Sci. 41:1546–1551. doi:10.2135/cropsci2001.4151546x

Shortall, O.K. 2013. "Marginal land" for energy crops: Exploring definitions and embedded assumptions. Energy Policy 62:19–27. doi:10.1016/j.enpol.2013.07.048

Uden, D.R., R.B. Mitchell, C.R. Allen, Q. Guan, and T.D. McCoy. 2013. The feasibility of producing adequate feedstock for year-round cellulosic ethanol production in an intensive agricultural fuelshed. BioEnergy Res. 6:930–938. doi:10.1007/s12155-013-9311-x

Veresoglou, S.D., A.P. Mamolos, B. Thornton, O.K. Voulgari, R. Sen, and D.S. Veresoglou. 2011. Medium-term fertilization of grassland plant communities masks species-linked effects on soil microbial community structure. Plant Soil 344:187–196. doi:10.1007/s11104-011-0739-5

Vogel, K.P., J.J. Brejda, D.T. Walters, and D.R. Buxton. 2002. Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. Agron. J. 94:413–420. doi:10.2134/agronj2002.0413

Wright, S. 1982. The shifting balance theory and macroevolution. Annu. Rev. Genet. 16:1–20. doi:10.1146/annurev.ge.16.120182.000245

Wullschleger, S.D., E.B. Davis, M.E. Borsuk, C.A. Gunderson, and L.R. Lynd. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. Agron. J. 102:1158–1168. doi:10.2134/agronj2010.0087

Yang, J., E. Worley, M. Wang, B. Lahner, D.E. Salt, M. Saha, and M. Udvardi. 2009. Natural variation for nutrient use and remobilization efficiencies in switchgrass. BioEnergy Res. 2:257–266. doi:10.1007/s12155-009-9055-9

Yost, M., N. Kitchen, K. Sudduth, A. Thompson, and E. Allphin. 2017. Topsoil thickness influences nitrogen management of switchgrass. BioEnergy Res. doi:10.1007/s12155-016-9811-6 (in press).