IMPACT OF RHIZOME QUALITY ON MISCANTHUS ESTABLISHMENT IN CLAYPAN SOIL LANDSCAPES

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ABSTRACT

Thousands of eroded-soil hectares in the U.S. Midwest have been planted to *Miscanthus × giganteus* as an industrial or bioenergy crop in recent years, but few studies on factors affecting crop establishment have been performed on these soils. The objective of this study was to quantify how both rhizome quality and depth of soil from the surface to the first argillic horizon (or depth to claypan (DTC¹)) affected *M. × giganteus* establishment. Rhizome quality (i.e., mass, length, diameter, viable buds, score), emergence, growth, and winter survival were measured on rhizomes planted in 2013 at Columbia and 2014 at Centralia, Missouri on clay loam soils with a range of DTC. Rhizome emergence and early tillering slightly increased as DTC increased, but these effects on growth diminished as the season progressed. Rhizome emergence and growth were more influenced by some metrics of rhizome quality; the odds of a rhizome emerging increased by 25 and 40% with each 1 cm and 1 bud increase in rhizome length and active bud count, respectively. Furthermore, late tiller counts, basal circumference, and end-of-season biomass increased as rhizome length and mass increased. Winter survival could not be estimated as well as emergence, but the odds of survival across sites increased by 5% with each 1 cm increase in rhizome length. When DTC was categorized as soil erosion class or landscape position, only the backslope at Centralia caused greater *M. × giganteus* growth than other positions. These findings demonstrate the resiliency of *M. × giganteus* for early growth and establishment on even the most degraded parts of the claypan soil landscape and indicate that propagating larger rhizomes will improve establishment.

Keywords: miscanthus establishment; rhizome quality; marginal soils; soil depth; landscape position

¹ DTC - depth to claypan or argillic horizon.
² EC_a – apparent electrical conductivity.
³ BCAP – Biomass Crop Assistance Program.

1. Introduction

Degraded soils, in traditional agricultural settings, are often typified as being less productive, and consequently, economically marginal. In addition, they are environmentally vulnerable, being more subject to erosion and topsoil loss than other soils (Scrivner et al., 1985; Kitchen et al., 1999; USDA-NRCS, 2006). One U.S. Midwest soil especially vulnerable to degradation is the Major Land Resource Area 113 Central Claypan Area (USDA-NRCS, 2006). Found throughout much of northeastern and central Missouri and central and southern Illinois, claypan soils are characteristically dense, compact, and contain a slowly permeable subsoil layer high in montmorillonitic clay referred to as the claypan. Due to their high clay content (often 500 to 650 g kg⁻¹) they generally maintain moisture well but have low plant-available water and limited or slow water infiltration. Erosion on these soils has been accelerated by decades of tillage-dependent grain cropping systems with relatively low productivity that provide no soil cover for 5 to 6 months each year (Massey et al., 2008). Consequently, claypan soils have low and sometimes negative profitability due to low grain yields (Massey et al., 2008). The challenges on these soils have motivated farmers to consider other crop options.

Miscanthus × giganteus is an emerging bioenergy and industrial crop that has high biomass production and high potential to remediate environmental problems associated with degraded soils (Lee et al., 2000; Bullard, 2001; Heaton et al., 2008; Thomas et al., 2014). Although best management practices of *M. × giganteus* have been studied for many areas in both the United States and Europe (Miguez et al., 2008; Xue et al., 2015), its production in the United States is limited. However, in 2012 the USDA implemented a national program through the Farm Service

Agency called the Biomass Crop Assistance Program (BCAP³) to provide financial assistance to growers seeking to establish and produce biomass feedstocks (USDA-Farm Service Agency, 2012). The program includes four project areas within the southern edge of the Midwest Corn (*Zea mays* L.) Belt. Of these, three were funded by the USDA through the former Midwest Farmers Association Oil Biomass (Columbia, Missouri) in 2012 to assist growers in establishing over 5,000 ha of *M. × giganteus* in central and southwestern Missouri, and in northeastern Arkansas. Within these three BCAP project areas, *M. × giganteus* was planted following cropland, pasture, and conservation reserve program land on soils that had historically experienced notable degradation and diminished grain crop productivity (Scrivner et al., 1985; Kitchen et al., 1999; USDA-NRCS, 2006).

A particular challenge experienced during the establishment of *M. × giganteus* in these three BCAP areas was poor rhizome emergence and growth accompanied with significant winterkill, which likely was caused by extreme weather conditions (i.e., spring downpours followed by historic droughts) and inadequate weed control. Poor emergence, growth, and winter survival resulted in crop failure in some fields and the need to replant. Replanting of rhizomes is expensive; planting material costs alone range between US \$1,240 to 2,700 ha⁻¹ compared to <\$740 ha⁻¹ for switchgrass (*Panicum virgatum* L.) (Anderson et al., 2015; Xue et al., 2015; personal communication with Renew Biomass). Planting costs are even higher for other methods of *M. × giganteus* propagation (i.e., rhizome-derived plants, stem nodal cuttings, or micropropagation) (Xue et al., 2015). The exception to these high costs might be sowing seed, as a new *M. × giganteus* cultivar that produces viable seed has been developed (Sacks et al., 2013; Anderson et al., 2015), but is not commercially available. Because propagation techniques are

difficult and costly (Christian and Haase, 2001), improved knowledge of factors affecting *M. × giganteus* establishment on degraded soils is critical for successful large-scale production.

Several interacting factors affect the successful propagation and establishment of *M. × giganteus*. Among others, these include soil properties and conditions, weather conditions before and after propagation, rhizome characteristics, and weed control (Heaton et al., 2004; Pyter et al., 2010). Several studies have investigated how many of these factors affect establishment of *M. × giganteus*. Results from many of these studies were recently reviewed and summarized along with surveys of seven miscanthus producers in Canada and Europe (Xue et al., 2015). This synthesis of research and experience identified rhizomes between 5 and 15 cm in length, 60 to 75 g in weight, or with at least three active buds as ‘best’ options for successful establishment (Xue et al., 2015). However, results among studies on *M. × giganteus* establishment show highly variable establishment efficiencies as a function of rhizome characteristics and soil and weather conditions. For example, emergence rates of propagated rhizomes have ranged from 8 to 100% and winter survival rates from 34 to 98% (Huisman and Kortleve, 1994; Christian and Haase, 2001; Christian et al., 2009; Pyter et al., 2009; Pyter et al., 2010; Boersma and Heaton, 2014). Therefore, more knowledge is needed about how soil conditions, rhizome or other propagation material characteristics, and weather conditions influence *M. × giganteus* establishment, especially on degraded soils.

The objectives of this study were to quantify how both rhizome characteristics or quality (i.e., mass, length, diameter, active buds, score) and DTC affect first-year *M. × giganteus* emergence, growth (i.e., tiller count, basal circumference, end-of-season biomass), and winter survival. The context of the investigation was to simulate rhizome and soil conditions representative of

production-scale *M. × giganteus* plantings found in the central Missouri and northern Arkansas BCAP project areas.

2. Materials and Methods

2.1. Site Descriptions

Two studies were conducted to accomplish the objectives of this investigation. The first during 2013 to 2014 was conducted at the University of Missouri South Farm located near Columbia, Missouri USA on a study site known as the Soil Productivity Assessment for Renewable Energy and Conservation (38°54' N, 92°16' W), and the second during 2014 to 2015 at a long-term USDA-Agricultural Research Service study site near Centralia, Missouri USA (39°13' N, 92°7' W). Both sites are within Major Land Resource Area 113 called the Central Claypan Area (USDA-NRCS, 2006) with predominately Mexico silt loam soil (fine, smectitic, mesic Aeric Vertic Epiaqualf).

2.2. Columbia, 2013

The research site at Columbia was a subset of plots (16 of 176) from a larger experiment that was established in 1982. The subset used included four replications of four soil erosion classes arranged in a completely randomized design. Each plot was 5.5 m wide × 9.4 m long (0.0049 ha). The erosion class treatments were initially created in 1982 by moving soil with land-leveling

tractors to represent various erosional phases or DTC typically observed on a claypan soil landscape. In 2009, these soil erosion classes were redefined using DTC as follows:

- (A) <5 cm of topsoil (severely eroded);
- (B) 5 to 20 cm of topsoil (moderately eroded);
- (C) 20 to 30 cm of topsoil depth (slightly eroded);
- (D) >30 cm of topsoil depth (non-eroded or depositional).

Depth to claypan was measured using apparent electrical conductivity (EC_a) values from a DUALEM-2S (Dualem Inc., Milton, ON, Canada) sensor mounted on a trailer and pulled behind an all-terrain vehicle. Three east-west transects of soil EC_a were obtained on all plots. On the same day as the EC_a survey, three 1.2-m deep soil samples from each plot were taken using a hydraulic coring machine (Giddings Machine Co., Windsor, Colorado, USA). The DTC was measured on each soil sample as the depth from the surface to the beginning of the argillic horizon. The soil EC_a at these sample locations was linearly correlated to the measured DTC to develop a calibration equation to convert all soil EC_a values to DTC, similar to procedures outlined in Kitchen et al. (1999) and Sudduth et al. (2010).

The prior cropping history of the 16 plots used in this investigation was corn and soybean (*Glycine max* [L.]) during 1982 to 1993, fallow with native grasses, legumes, and weeds during 1994 to 2008, switchgrass from 2009 to 2011, and failed *M. × giganteus* in 2012 attributed to excessively hot and dry weather conditions during the summer. In 2013, the present study was initiated by tilling the plots in the spring to provide a weed-free seedbed and planting *M. × giganteus* rhizomes by hand on 10 June 2013. Rhizomes were planted to a depth of 10 cm in six rows per plot with 76 cm spacing between rows and between plants within a row. Six of the 72 rhizomes used in each of the 16 plots ($n = 96$) were characterized with quality measurements

before planting. These six rhizomes were placed within and across rows and were monitored during 2013 to 2014. The rhizomes used were the Illinois clone, a cultivar certified by the Ohio Seed Improvement Association quality assurance program (USDA-Farm Service Agency, 2012), and were from the same source as those used in BCAP producer fields. They were mechanically harvested in January 2013 from a 2-yr-old dormant stand in southwest Missouri and kept refrigerated until planting.

Daily average air temperature, average sod soil temperature to 10 cm depth, and total precipitation were measured on-site. The 30-yr (1981-2010) normal average air temperature and total precipitation were obtained from the National Oceanic and Atmospheric Administration station in Columbia, Missouri.

2.2. Centralia, 2014

As was the case at the Columbia site, the Centralia site also utilized only a small subset of plots (3 of 30) from a larger experiment. The three large plots used in this study were each 18 m wide × 189 m long (0.34 ha) and were oriented on a west-facing slope so that each plot contained one replication of three non-randomized landscape position treatments of a typical claypan soil landscape. The three landscape position treatments in each plot were summit, backslope, and footslope as defined in 1991 by Kitchen et al. (1995) using DTC mapping and soil EC_a methods as previously described for the Columbia site.

The prior cropping history of the three plots used in this investigation was a corn-soybean rotation during 1991 to 2002, a corn-soybean-switchgrass rotation during 2002 to 2010, failed bioenergy willow (*Salix* spp.) in 2011 and 2012, cereal rye (*Secale cereale* L.) cover crop in fall

2012 to spring 2013, and failed *M. × giganteus* in 2013 attributed to cereal rye allelopathy. The present study was initiated in 2014. The plots were tilled with two passes of a disk cultivator to 13 cm depth before *M. × giganteus* rhizomes were planted on 21 May 2014 at 6,380 rhizomes ha⁻¹ by Midwest Farmers Association Oil Biomass using field-scale equipment across the entire plot areas to a depth of 10 cm. The rhizomes used were the same cultivar used at the Columbia site and were harvested in January 2014 from a 3-yr-old dormant *M. × giganteus* stand in Columbia, and kept refrigerated until planting. After the whole plot area was planted, 10 randomly selected rhizomes were hand-planted to a depth of 10 cm on each of the three landscape positions of each of the three plots ($n = 90$ rhizomes). Within each landscape position of each plot, the 10 selected rhizomes were spaced at ≥ 5 m apart across and within existing rows in order to represent a larger area of each landscape position. The physical locations of these 90 rhizomes were marked with flags and emergence, growth, and winter survival measurements were taken.

Daily average air temperature, average sod soil temperature to 10 cm depth, and total precipitation were measured on-site. The 30-yr (1981-2010) normal average air temperature was obtained from the National Oceanic and Atmospheric Administration station in Mexico, Missouri and total precipitation from the station in Centralia, Missouri.

2.3. Rhizome Quality

For both sites, the metrics of rhizome quality used were mass, length, diameter, and viable growth buds, and score. In order to ensure accuracy of measurements, all dormant rhizomes ($n = 90$ at Columbia and $n = 96$ at Centralia) were briefly rinsed in water to cleanse them of soil and debris. After cleaning, measurements and observations were obtained for mass, total length (the

sum of the lengths of all protruding parts), diameter, and apparent viable bud counts. For average rhizome diameter, a digital caliper was used to measure each rhizome at three random positions. From length and diameter measurements, rhizome volume was calculated. Rhizome volume divided by the number of viable buds was assigned a variable named 'rhizome score.' Monitored rhizomes with quality measurements used at Columbia ranged from 3 to 36 g in mass, 5 to 44 cm in length, 0.1 to 1.8 cm in diameter, 0 to 22 in active bud counts, and 0 to 6 in rhizome score. Likewise, the range at Centralia was 6 to 74 g mass, 5 to 30 cm length, 0.5 to 1.5 cm diameter, 1 to 23 active buds, and 0.4 to 13 score.

2.4. *Miscanthus* × *Giganteus* Growth Measurements

At both sites, each rhizome with quality measurements ($n = 96$ at Columbia and $n = 90$ at Centralia) was examined every other day following planting to record new stem or tiller emergence. As the growing season progressed and emergence of new tillers slowed, observations decreased to biweekly and then monthly intervals. At each observation, all tillers were counted regardless of height. Tiller count at 15 and 100 d after planting were selected as independent variables to describe early and late growth. Tiller height and diameter were not measured in this study due to difficulty in determining representative averages of these metrics for an individual plant with tillers of various sizes emerging at various times during first-year growth. At Columbia, all aboveground biomass >5 cm above the soil surface was harvested separately from each individual plant ($n = 96$) with hand pruners on 4 December 2013. At Centralia, to decrease injury to the plant from cutting too close to its base, all individual plants ($n = 90$) were harvested about 10 cm above the soil surface on 13 November 2014. All harvested biomass from each

plant at both sites was dried at 60°C for 72 hr then weighed to determine dry matter yield per plant. Immediately before harvest at both sites, basal circumference also was obtained by measuring with a seamstress tape around the circumference of the clump of emerged tillers as close to the soil surface as possible. This measurement was possible because characterized plants were adequately spaced from surrounding plants and spread was minimal during first-year growth.

Rhizomes with no tillers from planting through harvest were considered non-emergent. For each rhizome that did emerge, winter survival was determined by assessing the tillering and growth of rhizomes the following spring and summer. Rhizomes with ≥ 1 living tiller were categorically rated as survival. Rhizome emergence percentage by soil erosion class at Columbia or landscape position at Centralia was determined as the number of live rhizomes divided by the total number of rhizomes that were planted. Likewise, winter survival percentage was the number of live rhizomes after the winter divided by the number that emerged.

2.5. Statistical Analyses

To determine the fixed, categorical effects of landscape position (summit, backslope, and footslope) of the Centralia study and soil erosion class (A, B, C, and D) of the Columbia study on *M. × giganteus* growth and establishment, data were analyzed at $P \leq 0.05$ by study site using ANOVA with the MIXED procedure of SAS (SAS Institute, 2006). The dependent variables at both sites were emergence percentage, rhizome tiller count 15 and 100 d after planting, plant basal circumference, end-of-season biomass, and winter survival percentage. The independent variables were landscape position at Centralia and soil erosion class at Columbia. The

assumptions of normality and equal variance were satisfied by examining residuals with the UNIVARIATE procedure of SAS. Mean separations were conducted using Fisher's protected LSD (0.05).

To determine which continuous effects of rhizome quality measurements and DTC influenced *M. × giganteus* emergence, growth, and winter survival data were analyzed by and across sites using multiple regressions. For the binary dependent variables of emergence (no emergence = 0, emergence = 1) and winter survival (no survival = 0, survival = 1), the LOGISTIC procedure of SAS was used. Potential independent variables were the linear and quadratic form of DTC and rhizome mass, length, diameter, score, viable bud count, along with all two-way interactions of the linear forms of these variables. For the continuous rhizome growth measurements (rhizome tiller count 15 and 100 d after planting, plant basal circumference, and end-of-season biomass), the REG procedure of SAS was used with the same set of potential independent variables as emergence and winter survival. All multiple regression models used stepwise selection of independent variables with an entry level of $P \leq 0.10$ and an exit level of $P \geq 0.15$ (Hosmer et al., 2103). Lower-order models (i.e., without squared and/or interaction terms) were tested and used when they had measures of association or correlation coefficients that were equal to or only slightly lower than higher-order models. Multicollinearity among predictors was examined using the variance inflation factor option in the REG procedure of SAS (Allison, 2012).

3. Results and Discussion

3.1. Weather Conditions

Average monthly soil temperatures during the growing-season (April – October) were similar across the two site-years with the exception of warmer temperatures (+3°C) in July and September at Columbia in 2013 than Centralia in 2014 (Table 1). Average growing-season air temperature was within 1°C of the 30-yr (1981-2010) normal at both sites. The most significant deviations from normal air temperature (-3°C) were in April 2013 at Columbia and July 2014 at Centralia. Growing-season total precipitation was 12% below the 30-yr normal of 808 mm at Columbia in 2013. However, early spring (April – May) precipitation was much higher than normal (202 mm above) followed by a dry summer and autumn (294 mm below). At Centralia in 2014, growing-season total precipitation was more evenly distributed across the season than at Columbia in 2013 and seasonal precipitation in 2014 was 14% above the 30-yr normal of 752 mm.

3.2. Emergence

The first metric of *M. × giganteus* establishment is successful emergence or the ability of the propagule to produce tillers. As noted by Boermisa and Heaton (2014), this aspect of establishment largely has been overlooked in the literature. In the present study, neither soil erosion class at Columbia nor landscape position at Centralia affected the percentage of rhizomes that emerged (i.e., produced ≥ 1 tiller plant⁻¹ between planting and harvest) (Table 2). However, it should be noted that numeric differences were apparent between the mean emergence rate for the most eroded class and other less eroded classes at Columbia (class A was 8% higher than other classes) and Centralia (backslope was 20% higher than other positions).

Across classes at Columbia, 8 of 96 planted rhizomes (8%) did not emerge compared to 14 of 90 rhizomes (16%) across landscape positions at Centralia. Thus, the emergence failure rate was twice as great at Centralia than Columbia. The cause of more failure at Centralia may have been due, in part, to less precipitation prior to planting and cooler soil temperatures after planting. Although Centralia had much more (349 mm) precipitation during June through October than Columbia, it had 190 mm less precipitation during April through May (Table 1). In addition, soil temperatures were 1 to 4°C lower during June through July at Centralia. Across sites, 88% of the planted rhizomes successfully emerged. This rate was comparable to the emergence rate of *M. × giganteus* grown in Iowa, USA (Boersma and Heaton, 2014).

Multiple logistic regression models were developed to identify whether combinations of rhizome characteristics and DTC relate to emergence of individual rhizomes. At Columbia, the odds of a rhizome emerging increased by 1.593-fold (59.3%) with each 1 cm increase in rhizome length and by 1.577-fold (57.7%) with each 1 bud increase in active bud count (Table 3). This model was able to correctly identify 84% of the emerged rhizomes as emerged and 100% of the non-emerged as such for data used to create the model. This and all other multiple regression models developed in this study were not cross-validated because sample sizes were small (i.e., only 186 rhizomes representing two site-years). Therefore, the multiple regression results presented here and throughout should not be considered quantitatively predictive, but rather as potential indicators of which and to what relative extent rhizome and soil characteristics might influence *M. × giganteus* emergence, growth, and winter kill. To this end, the selected variables in this and following models and their directional effects on independent variables align well with common agronomic knowledge. For example, previous studies have noted that rhizome emergence improves when larger rhizomes are used (reviewed in Xue et al., 2015). Furthermore,

creating and comparing regression models by and across the two sites helped identify common potential predictors of rhizome establishment.

Rhizome emergence results at Centralia were similar to those at Columbia; the odds of a rhizome emerging increased by 14.3% with each 1 cm increase in rhizome length, by 33.7% with each 1 bud increase in active bud count, and by 8.0% with each 1 cm increase in DTC (Table 3). This model was able to correctly identify 76 and 71% of the rhizomes as emerged and nonemerged, respectively, for data used to create the model. Compared to the Columbia site, the additional influence of DTC and the much lower influence of rhizome length and active bud count on emergence at Centralia may be related to less ideal soil conditions at Centralia near planting (i.e., less precipitation and cooler soil temperatures than normal). Furthermore, quantitative DTC measurements (Table 3) were more indicative of rhizome emergence than general landscape position class (Table 2) at Centralia, likely because DTC is a better estimator of unique soil properties (e.g., potential plant available water content). These results highlight a potential interaction between soil conditions at planting and rhizome characteristics on rhizome emergence, which would complicate the process of identifying optimal rhizome quality characteristics across many environments. However, the fact that both rhizome length and active bud count were selected and influenced emergence in the same direction at each independent site-year indicates that these two variables may help predict emergence.

When the analysis was conducted across sites, only the same two rhizome quality measurements (i.e., length and active bud count) selected at individual sites were included. The selected model indicated that the odds of a rhizome emerging increased by 25.3% with each 1 cm increase in rhizome length and 39.5% with each 1 bud increase in active bud count. This combined model correctly identified 77 and 73% of the rhizomes as emerged and nonemerged,

respectively for data used to create the model. Therefore, results across site-years confirmed those by site-year and intuitively indicate that increasing the length and active bud count number of rhizomes used for propagation should greatly improve emergence success.

3.3. Growth

3.3.1. Tillering

Once a *M. × giganteus* rhizome emerges, growth of tillers during the summer and fall influence the plant's ability to survive the first winter and successfully establish. To examine early and late growth, tiller counts were conducted. The soil resource (i.e., soil erosion class) at the Columbia site had no impact on tiller count 15 or 100 d after planting (Table 2). Across erosion classes, tiller count averaged 1.5 and 19.9 tillers plant⁻¹ at 15 and 100 d after planting, respectively. Tiller counts were similar at Centralia in 2014, but in contrast to results from Columbia, both counts were highly affected by landscape position. Tiller count 15 d after planting was 0.7 tillers plant⁻¹ (70%) greater at the backslope than the summit, and tiller count at the footslope was equal to both the backslope and the summit. Landscape position effects on tiller count were greater at 100 d after planting. The backslope had 7.7 tillers plant⁻¹ (64%) more tillers than the other two landscape positions. The unique impacts of landscape position on *M. × giganteus* tillering and other growth measurements will be discussed together in detail below in section 3.3.3.

Multiple linear regressions were used to identify combinations of rhizome characteristics and DTC that might influence *M. × giganteus* tillering by and across sites for rhizomes that emerged

(Table 4). At the Columbia site, tiller count 15 d after planting increased by 0.00198 and 0.00138 tillers plant⁻¹ with each one unit increase in the product of DTC × viable bud count and of rhizome mass × length, respectively. Thus, as an example scenario, a rhizome with 2 active buds, 25 g in mass, and 20 cm in length planted on soil with a 15 cm DTC would have an estimated 1.6 tillers at 15 d after planting ($0.87 + [15 \text{ cm DTC} \times 2 \text{ buds} \times 0.00198] + [25 \text{ g mass} \times 20 \text{ cm length} \times 0.00138]$). This model accounted for 27% ($r^2 = 0.27$) of the variability in tiller count 15 d after planting and indicated that early tillering may be enhanced when rhizomes are larger, have more active buds, and are grown on soils with greater DTC. Rhizome bud count is a clear indicator of rhizome vitality in the form of an already manufactured and ready-to-go growing component and appears to be important early in the season for improved emergence and tillering. However, viable bud count did not relate to tiller count 100 d after planting (Table 4), which indicates that bud count effects may be masked later in the season as tiller count and growth increase and are able to regenerate the rhizome. The only variable accounting for variation in late tiller counts was rhizome length, but it only accounted for 6% of the variation in late tillering counts (Table 4; Fig. 1). The lack of DTC effects on late tillering supports the results above and implies that soil conditions may be more important for emergence and early growth than later growth and winterkill.

At Centralia in 2014, tiller count 15 and 100 d after planting increased by 0.039 and 0.377 tillers plant⁻¹ with each 1 g increase in rhizome mass, respectively. These models accounted for 20 to 22% of the variability in tiller counts (Table 4). These results indicate that rhizomes with greater mass may improve *M. × giganteus*'s ability to tiller and grow during the establishment year. Individual results from Columbia and Centralia demonstrate that site-year influenced which metrics of rhizome quality and DTC affect early and late tillering during *M. × giganteus*

establishment. At Columbia, rhizome length was the only variable that accounted for variation in early and later tillering, whereas at Centralia, it was rhizome mass. When the sites were analyzed together, the variables selected for tillering were the same as those at Columbia alone (Table 4). In the model for tiller count at 15 d after planting across sites, the linear coefficient or slopes of $\text{DTC} \times \text{buds}$ and $\text{rhizome mass} \times \text{length}$ decreased by 3 and 12%, respectively, compared to the same slopes for Columbia alone. Conversely, the slope of rhizome length for tiller count 100 d after planting increased by 42%. Several contrasting features between the two site-years, including differences in weather, DTC, and the absence of landscape slope at Columbia, may explain why the results for tillering were different. That said, it appears that early and late rhizome tillering may be more influenced by site-specific factors than rhizome emergence.

3.3.2. Basal Circumference

Plant basal circumference at harvest, an indicator of how the plant has spread over the growing season, was not influenced by soil erosion class at Columbia, but was by landscape position at Centralia (Table 2). The average basal circumference at harvest was 62.2 cm across erosion classes at Columbia and was 61.1 cm at the backslope vs. an average of 41.1 cm across the summit and footslope at Centralia. Greater basal circumference at the backslope aligns with the results of greater tiller count 15 and 100 d after planting at this position and indicates greater *M. × giganteus* spreading on the most eroded soils at Centralia. This phenomenon was unexpected and likely related to the microenvironment of the backslope position, as discussed in greater detail below in section 3.3.3.

Multiple linear regression models were used to identify which rhizome characteristics and DTC might influence first-year *M. × giganteus* basal circumference at harvest by and across sites for rhizomes that emerged (Table 4; Fig. 1). At Columbia, none of the DTC or rhizome quality variables influenced basal circumference. At Centralia, basal circumference increased by 0.564 cm with each 1 cm increase in rhizome mass, but the correlation was weak ($r^2 = 0.05$). Similarly, across sites, basal circumference increased by 0.577 cm with each 1 cm increase in rhizome length, and the correlation remained weak ($r^2 = 0.09$). Therefore, rhizomes with greater length and mass may sometimes increase basal circumference, but other factors not measured in this study appear to have a greater influence. Among others, these factors likely include interactions between rhizome characteristics [size, planting orientation (vertical or horizontal), and physical location of active buds on the rhizome] and soil physical properties (e.g., aggregation and bulk density). Future studies should address whether these factors affect first-year *M. × giganteus* growth and establishment.

Linear correlations between basal circumference, tiller counts 100 d after planting, and end-of-season biomass were examined for rhizomes that emerged. Across sites, tiller count after 100 d increased by 0.31 tillers plant⁻¹ with each 1 cm increase in basal circumference ($y = 0.661 + 0.31x$, $P < 0.001$, $r^2 = 0.57$). Furthermore, end-of-season biomass increased by 0.98 g with each 1 cm increase in basal circumference ($y = 4.25 + 0.98x$, $P < 0.001$, $r^2 = 0.21$). The stronger correlation between basal circumference and tiller count 100 d after planting than end-of-season biomass suggests that larger basal circumference usually lends to more tillers, but does not always increase biomass. High tiller density could produce a smaller basal circumference but still have a higher yield than a larger circumference due to the number of tillers. Conversely, the lack of high correlations among two-way comparisons of these three growth metrics indicates that

they are unique and that they may be individually important for assessing first-year *M. × giganteus* growth.

3.3.3. End-of-Season Biomass

The final growth metric used in this study, end-of-season biomass, which averaged 42.5 g plant⁻¹, was not impacted by soil erosion class at Columbia (Table 2). However, landscape position at Centralia did influence end-of-season biomass. Biomass at the backslope landscape position (102.1 g plant⁻¹) was 78% higher than that measured on the summit and footslope positions (57.5 g plant⁻¹). Greater *M. × giganteus* growth (tiller counts, basal circumference, and end-of-season biomass) at the backslope was not expected because eroded backslopes can sometimes produce less grain crop yield and switchgrass yield than other landscape positions (Thelemann et al., 2010; Yost et al., 2015) with deeper topsoil and higher plant available water (Jiang et al., 2007). Furthermore, switchgrass establishment (i.e., stand counts or frequency measurement) was not influenced by landscape position in Iowa, USA (Wilson et al., 2014).

We speculate that emergence and growth was greater at the backslope than the summit because the latter is exposed to more rapid wind speeds at this site that may have increased water stress during dry periods (Logsdon et al., 2014). This result was substantiated by observations at this site of lower willow yield on the summit than backslope in 2011 and 2012. *Miscanthus × giganteus* emergence and growth on the backslope may have outperformed the footslope due to warmer soil temperatures (Table 1) caused by lower water supply (Logsdon et al., 2014). As the first report on replicated impacts of landscape position on *M. × giganteus* establishment, our results suggest that emergence and first-year growth may be enhanced in shallower soils. This

would indicate that shallow, eroded soils could be a prime target for *M. × giganteus*. However, these unexpected results should be further validated.

Multiple linear regressions results for Columbia indicated that as rhizome length increased so did biomass. Biomass increased by 1.41 g plant⁻¹ with each 1 cm increase in rhizome length and length accounted for 16% of the variation in biomass (Table 4, Fig. 1). At Centralia, biomass increased by 2.38 g plants⁻¹ with each 1 g increase in rhizome mass and mass accounted for 20% of the variation. Thus, for end-of-season biomass and for the other two growth measurements, rhizome length and mass were the two metrics that most consistently related to *M. × giganteus* growth at these two sites. Though these two metrics are somewhat related ($r^2 = 0.41$, length = $8.04 + 0.52$ mass, $P < 0.001$), a plausible explanation of their importance is that they may represent the rhizome carbohydrate storage needed for initial growth and establishment. Furthermore, greater rhizome length may provide greater surface area for contact with the soil to initiate growth. The striking difference in the potential relative importance of rhizome length at Columbia vs. rhizome mass at Centralia was probably related to the ranges of these measurements; Columbia had some heavier rhizomes and Centralia some longer rhizomes. However, when analyzed across site-years, biomass increased by 2.42 g plant⁻¹ with each 1 g increase in rhizome mass with a slightly improved correlation ($r^2=0.22$) above individual sites. Therefore, rhizome mass may be more closely related to first-year rhizome growth than rhizome length.

In general, the selected correlations among rhizome quality metrics (length, mass, active bud count) and *M. × giganteus* growth (tillering, basal circumference, and end-of-season biomass) were relatively weak ($r^2 \leq 0.23$) (Table 4; Fig. 1). Furthermore, some metrics of rhizome quality (i.e., rhizome diameter and score) had no apparent effect on

early *M. × giganteus* growth within or across sites. Rhizome characteristics likely do not tightly correlate to first-year *M. × giganteus* growth because the vigor and growth of a plant, once rhizome shoots have emerged and new photosynthetic capacity is established, may over time diminish their effects. If a plant successfully emerges, the local environmental conditions around the plant (e.g., soil moisture, nutrients, and weed competition) could have greater impact on *M. × giganteus* growth than rhizome quality. Despite the inability of rhizome characteristics to precisely estimate first-year *M. × giganteus* growth, these results clearly show that larger (longer and/or more massive) rhizomes commonly result in improved emergence and first-year growth of *M. × giganteus* on claypan soils, as evidenced by others on nonclaypan soils (Jørgensen, 1995; Christian and Haase, 2001; Pyter et al., 2010).

3.5. Winter Survival

Rhizome winter survival is the key metric for successful *M. × giganteus* establishment. If rhizomes do not survive the first winter, their productivity during the first season is of no significance. Rhizome winter survival percentages were low at both sites, but were much lower at Centralia than Columbia. At the Columbia site, 64% of the 88 planted rhizomes that produced tillers throughout the initial growing season overwintered. At Centralia, only 29% of the 76 planted rhizomes that produced tillers overwintered. Thus, the winter survival of emerged rhizomes was slightly over twice as high at Columbia than Centralia. Lower survival at Centralia was, in part, related to more intensive weed control at this location that either weakened *M. ×*

giganteus plants and/or eliminated nearly all weed biomass that would have provided soil cover during the winter.

Several other factors influence the ability of *M. × giganteus* to survive the first winter after establishment. Two major factors are air and soil temperatures during the winter and early spring following establishment (Hasting et al., 2009). Several previous studies have reported lethal *M. × giganteus* rhizome kill (>50%) during the first winter if soil temperatures drop below about -3.4 to -4.0°C (Clifton-Brown and Lewandowski, 2000; Clifton-Brown et al., 2001; Kucharik et al., 2013; Friesen et al., 2015; Peixoto et al., 2015; Sage et al., 2015). However, some reports indicate that first-year *M. × giganteus* stands have consistently withstood harsher winter conditions; air temperatures as cold as -20°C and soil temperatures as low as -6°C (Heaton et al., 2010; Friesen et al., 2015). In the present study at the Columbia site, average monthly soil temperatures at a 10-cm depth were $\geq -1.3^{\circ}\text{C}$ through much of the winter and early spring (November 2013 – December 2013 and February 2014 – April 2014), but reached as low as -5.8°C in January. Furthermore, average monthly air temperatures during the winter and early spring were $> -4^{\circ}\text{C}$, but reached as low as -24°C in January. Although cold soil temperature in January may have increased winterkill at this site, >50% of the plants survived the first winter, which suggests lethal soil temperatures below -5.8°C as evidenced in Illinois, USA and Ontario, CAN (Heaton et al., 2010; Friesen et al., 2015). At Centralia, average air and soil temperatures during the winter and early spring were generally warmer than at Columbia and the lowest air (-15°C) and soil temperatures (-2°C) occurred during January and February. Therefore, weather conditions experienced at Centralia likely did not influence the low winter survival.

Another factor that affects *M. × giganteus* winter survival is its ability to store or sequester a critical amount of metabolic reserve going into the first winter (Christian and Hasse, 2001; Purdy

et al., 2015). This ability to store metabolites likely is influenced by the quality of the planted rhizome and the soil resource that supports the rhizome. However, neither soil erosion class at Columbia nor landscape position at Centralia affected winter survival percentage rates of emerged rhizomes (Table 2). Therefore, greater emergence and productivity on the backslope at Centralia (Table 2, 3) did not equate to increased rhizome winter survival percentage, suggesting a weak correlation between first-year growth and winter survival. Conversely, the lack of reduced winter survival on eroded backslopes indicates that *M. × giganteus* could be grown on eroded soils without compromising its ability to survive the first winter.

Winter survival at Columbia also may have been slightly reduced due to the lower cutting of the biomass in the fall (5 cm above soil surface instead of 10 cm at Centralia) that could have made the young plants more vulnerable. This assessment is supported by the fact that winter survival of the adjacent plants, not a part of this rhizome study and that were not harvested, had a 14% higher winter survival rate. It is also supported by observations made by former Midwest Farmers Association oil biomass agronomists in some BCAP fields where removing first-year biomass increased winterkill, likely due to cooler soil temperatures resulting from no residue cover (Kucharik et al., 2013). Based on these results and observations, any removal of first-year biomass for growth measurements may negatively influence winter kill assessments. Therefore, in future studies where both first-year growth and winterkill are measured, it may be necessary to cut end-of-season biomass at greater heights above the soil surface and return residue for cover after measurements are taken.

Multiple logistic regression models were developed to determine whether rhizome quality measurements and DTC affect winter survival of individual rhizomes (Table 5). Results by site showed that rhizome length influenced winter survival at Columbia, but neither rhizome quality

measurements nor DTC affected survival at Centralia. At Columbia, the odds of a rhizome surviving the winter increased by 8.0% with each 1 cm increase in rhizome length and length correctly identified survival or non-survival of 55% of the emerged rhizomes for data used to build the model. Across sites, rhizome length was important for estimating the odds of rhizome survival, but the odds ratio of length decreased from 8% for Columbia alone to 5% for both sites, and 52% of the rhizomes were correctly classified as survival or non-survival. These results align with others who have shown that larger (including longer) rhizomes with potentially more nutrient reserves going into winter can sometimes improve rhizome winter survival (Pyter et al., 2010; Xue et al., 2015). However, additional factors other than rhizome size that were not measured in this study also influence winter survival. Identifying these factors is beyond the scope of this investigation, but they likely include interactions among rhizome quality, rhizome storage time before planting (Pyter et al., 2010; Xue et al., 2015), first-year growth and rhizome nutrient storage going into winter (Pyter et al., 2009), and timing between plant senescence and killing frost and soil temperatures during winter (Pexioto et al., 2015).

This approach of using multiple logistic regression could be applied to larger datasets across many environments to better estimate rhizome, soil, and weather variables influencing first-year winter survival of *M. × giganteus*. Furthermore, these investigations should include a larger range of rhizomes sizes than used in this study. The range in this study was lower than another North American study (Pyter et al., 2010) because rhizomes were harvested mechanically from young parent plants (2- to 3-yr-old), which resulted in smaller rhizome cuttings.

4. Conclusions

This investigation was the first of its kind to quantify how rhizome quality metrics of mass, length, diameter, viable bud counts, and score, along with variable soils found across a claypan soil landscape, can affect the emergence, first-year growth, and winter survival of *M. × giganteus*. The information obtained for rhizome emergence was especially important because few results on emergence exist in the literature. Rhizome emergence was influenced by soil erosion class at Columbia (numerically not statistically) and landscape position at Centralia (statistically); with the shallowest soil at both locations causing the highest emergence. After emergence, only landscape position at Centralia influenced first-year *M. × giganteus* growth with the shallowest soil (i.e., backslope) again performing the best. This phenomenon of improved emergence and growth on shallower soils may be related to the microenvironment of the backslope position at Centralia and/or to other factors not measured in this study. Although these results need to be validated in future studies, it is encouraging that *M. × giganteus* emergence and first-year growth appears to be not highly sensitive to these degraded portions of the landscape.

Rhizome emergence results also indicated that longer rhizomes with more active buds may improve emergence success, and that the soil resource (i.e., DTC) may be important for emergence when less than ideal planting conditions occur (i.e., low precipitation and cool temperatures). Shortly after emergence, early tillering may be influenced by DTC, active bud counts, rhizome mass, and rhizome length. However, for later tillering and growth measurements, the effects of DTC and active bud count appeared to diminish, while mass and length continued to have a positive effect on growth metrics. Therefore, larger rhizomes should be used on claypan soils because they improved *M. × giganteus* emergence and early growth.

The ultimate measure of *M. × giganteus* establishment success, winter survival, was the most difficult to estimate with rhizome quality and soil resource metrics used in this study. The only variable selected to account for some variation in winter survival was rhizome length; longer rhizomes increased the odds of winter survival. This rhizome quality metric was the most frequently selected variable among all the regression models developed by and across sites for rhizome emergence, growth, and winterkill. Therefore, rhizome length may potentially be one of the best rhizome quality metrics to use as an overall indicator of successful *M. × giganteus* establishment. However, its significance and relevance should be validated in future research. Furthermore, the approach of using multiple regressions to identify rhizome and soil factors that influence *M. × giganteus* emergence, growth, and winter survival rates could be used on larger datasets across many environments so that site-specific agronomically and economically optimum sizes of rhizomes to use for propagation could be established.

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Table 1

Average soil and air temperatures, and total precipitation with differences from the 30-yr normal (1981-2010) in parenthesis for the University of Missouri South Farm site located near Columbia, Missouri and the Centralia Research Farm site located near Centralia, Missouri.

Month	Columbia				Centralia			
	Year	Soil ^a	Air	Precip.	Year	Soil ^a	Air	Precip.
		—— °C ——		— mm —		—— °C ——		— mm —
April	2013	11	11 (-3)	178 (64)	2014	11	12 (0)	169 (66)
May		17	17 (-1)	277 (138)		18	19 (1)	81 (-42)
June		23	22 (-1)	54 (-78)		23	23 (0)	139 (8)
July		26	24 (-2)	35 (-80)		23	22 (-3)	56 (-51)
August		25	24 (-2)	48 (-67)		25	24 (-1)	146 (44)
September		23	21 (1)	51 (-58)		20	19 (-1)	65 (-35)
October		15	13 (-1)	73 (-11)		14	13 (0)	204 (118)
November		6	5 (-3)	38 (-68)		5	3 (-4)	19 (-59)
December		1	-1 (-2)	40 (-24)		4	2 (2)	18 (-40)
January	2014	-1	-4 (-3)	8 (-106)	2015	0	-1 (1)	24 (-19)
February		-1	-4 (-6)	9 (-130)		1	-5 (-5)	19 (-32)
March		4	5 (-3)	26 (-106)		5	7 (1)	49 (-21)
April		12	13 (-1)	161 (46)		13	14 (2)	64 (-39)

^a Measured at a depth of 10 cm on sod soil.

Table 2

Mean *M. × giganteus* rhizome tiller count 15 or 100 d after planting, plant circumference at harvest, end-of-season biomass, and winter survival percentage by soil erosion class for Columbia, Missouri in 2013 or by landscape position for Centralia, Missouri in 2014 only for rhizomes that emerged.

Site (yr)	Dependent variable	Units	$P > F$	Erosion class or landscape position ^a	LS mean ^b
Columbia (2013)	emergence	% of planted rhizomes	0.88	A	96a
				B	88a
				C	88a
				D	88a
	tillers after 15 d	tillers plant ⁻¹	0.56	A	1.4a
				B	1.7a
				C	1.3a
				D	1.6a
	tillers after 100 d	tillers plant ⁻¹	0.39	A	22.3a
				B	19.5a
				C	17.7a
				D	20.1a
	basal circum.	cm	0.14	A	70.3a
				B	57.2a
				C	58.0a
				D	63.2a
	end-of-season biomass	g plant ⁻¹	0.40	A	51.1a
				B	47.4a
				C	37.8a
				D	50.9a
	winter survival	% of emerged rhizomes	0.12	A	79a
				B	66a
				C	46a
				D	65a
Centralia (2014)	emergence	% of planted rhizomes	0.34	summit	77a
				backslope	97a
				footslope	77a
	tillers after 15 d	tillers plant ⁻¹	0.011	summit	1.0b
				backslope	1.7a
				footslope	1.3ab
	tillers after 100 d	tillers plant ⁻¹	<0.001	summit	10.3b
				backslope	19.8a
				footslope	13.9b
	basal circum.	cm	<0.001	summit	37.4b
				backslope	61.1a
				footslope	44.8b
	end-of-season biomass	g plant ⁻¹	<0.001	summit	44.1b
				backslope	102.1a

winter	% of	0.36	footslope	70.6b
survival	emerged		summit	24a
	rhizomes		backslope	23a
			footslope	40a

^a Soil classes are defined as: A, <5 cm of topsoil (severely eroded); B, 5 to 20 cm of topsoil (moderately eroded); C, 20 to 30 cm of topsoil depth (slightly eroded); D, >30 cm of topsoil depth (non-eroded). Landscape positions are defined as: summit (slightly eroded), backslope (severely eroded), and footslope (non-eroded or depositional).

^b Within site and dependent variable, means with the same letter were not statistically different at LSD (0.05).

775 **Table 3**
776 Parameter estimates with associated probabilities of chi-squared tests, odds ratios with 95% Wald
777 confidence intervals (CI), and measures of association for multiple logistic regression models used to
778 predict the occurrence of rhizome emergence.

Site (yr)	Parameter	Parameter estimate	Odds ratio	95% CI	Measures of association ^a	
					Concordant (discordant) pairs	Somers' D
Columbia (2013)	intercept	-4.869**			%	
	length	0.466**	1.593	(1.097, 2.315)	94 (6)	0.89
	buds	0.456**	1.577	(1.035, 2.403)		
Centralia (2014)	intercept	-2.759**			80 (19)	0.61
	length	0.134*	1.143	(0.971, 1.345)		
	buds	0.291**	1.337	(1.026, 1.744)		
	DTC ^b	0.0771*	1.080	(0.993, 1.175)		
Columbia, Centralia (2013,14)	intercept	-2.546***			84 (15)	0.69
	length	0.226***	1.253	(1.079, 1.456)		
	buds	0.333***	1.395	(1.125, 1.729)		

779 * Significant at $P \leq 0.15$.

780 ** Significant at $P \leq 0.05$.

781 *** Significant at $P \leq 0.01$.

782 ^a Concordance occurred when emerged rhizomes were correctly classified as emerged and discordance
783 occurred nonemerged rhizomes were classified as emerged; tied pairs are not shown. Somers' D = (no
784 of concordant pairs – no of discordant pairs) / total no. of pairs.

785 ^b DTC, depth to claypan.

Table 4

Multiple linear regression results from two research sites where rhizome quality metrics and depth to claypan were used to predict the response of rhizome tillers after 15 and 100 d of planting, plant basal circumference at harvest, and end-of-season biomass.

Site (yr)	Dependent variable (y)	y units	Regression equation ^a	Model significance	r^2
Columbia (2013)	tillers after 15 d	tillers plant ⁻¹	$y = 0.87 + 0.00198cb + 0.00138ml$	<0.001	0.27
	tillers after 100 d	tillers plant ⁻¹	$y = 15.3 + 0.277l$	0.013	0.06
	basal circumference	cm	$y = 62.3$	0.061	-
	end-of-season biomass	g plant ⁻¹	$y = 23.5 + 1.41l$	<0.001	0.16
Centralia (2014)	tillers after 15 d	tillers plant ⁻¹	$y = 0.68 + 0.039m$	<0.001	0.22
	tillers after 100 d	tillers plant ⁻¹	$y = 8.6 + 0.377m$	<0.001	0.20
	basal circumference	cm	$y = 39.1 + 0.564m$	0.024	0.05
	end-of-season biomass	g plant ⁻¹	$y = 33.8 + 2.38m$	<0.001	0.20
Columbia, Centralia (2013,14)	tillers after 15 d	tillers plant ⁻¹	$y = 0.84 + 0.00192cb + 0.00122ml$	<0.001	0.23
	tillers after 100 d	tillers plant ⁻¹	$y = 11.5 + 0.394l$	<0.001	0.09
	basal circumference	cm	$y = 47.0 + 0.577l$	0.022	0.03
	end-of-season biomass	g plant ⁻¹	$y = 25.8 + 2.42m$	<0.001	0.22

^a y represents the dependent variable. Lower case letters represent independent variables selected by stepwise selection. Independent variables include: *c*, depth to claypan (cm); *m*, rhizome mass (g); *l*, rhizome length (cm); *b*, number of viable buds on rhizome. All reported parameters are significant at $P \leq 0.10$.

793 **Table 5**
794 Parameter estimates with associated probabilities of chi-squared tests, odds ratios with 95% Wald confidence
795 intervals (CI), and measures of association for multiple logistic regression models used to predict the
796 occurrence of rhizome winter survival.

Site (yr)	Parameter	Parameter estimate	Odds ratio	95% CI	Measures of association ^a	
					Concordant (discordant) pairs	Somers' D
					%	
Columbia (2013)	intercept	-0.713			60 (36)	0.24
	length	0.0773**	1.080	(1.010, 1.156)		
Centralia (2014)	intercept	-0.898	-	-	-	-
Columbia, Centralia (2013,14)	intercept	-0.942**			55 (41)	0.14
	length	0.0523**	1.054	(1.007, 1.102)		

797 * Significant at $P \leq 0.10$.

798 ** Significant at $P \leq 0.05$.

799 *** Significant at $P \leq 0.01$.

800 ^a Concordance occurred when living rhizomes were correctly classified as living and discordance occurred dead rhizomes were
801 classified as living; tied pairs are not shown. Somers' D = (no of concordant pairs – no of discordant pairs) / total no. of pairs.

802

803 **Fig. 1.** Linear correlations between tiller count 100 d after planting, basal circumference, and
804 end-of-season biomass and rhizome length at Columbia, 2013 and rhizome mass at Centralia,
805 2014.
806

Figure