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Application Timing of Herbicides for Miscanthus (Miscanthus X Giganteus) Control and Effects of Mowing on Rhizome Initiation and Production

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Application timing of herbicides for Miscanthus (*Miscanthus x giganteus*) control and effects of mowing on rhizome initiation and production

By

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Application timing of herbicides for Miscanthus (*Miscanthus x giganteus*) control and effects of mowing on rhizome initiation and production

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Herbicide treatments were tested on mature stands of *Miscanthus* in 2013 and 2014 in Winston and Oktibbeha counties Mississippi. Twenty-one different herbicide treatments and two application timings, summer and fall, were evaluated. Glyphosate at 4,500 g ae ha^{-1} applied in the summer provided the best *Miscanthus* control at each location. Control with fall applications of glyphosate varied between locations.

Two greenhouse studies were conducted in 2014 and 2015 at Mississippi State, MS to evaluate the effects of mowing on seedling *Miscanthus*, as well as the time period between seed germination and rhizome initiation. Rhizomes were visible on seedling plants 15 or 13 weeks after germination in 2014 and 2015, respectively. Removal of the *Miscanthus* terminal reduced the number of rhizomes produced compared to plants with intact terminals. However, terminal removal increased the number of shoots produced compared to plants with intact terminal.

DEDICATION

I would like to dedicate this work to my husband, Blake, and my children, Cade and Issac. They were my inspiration to continue forward even when I doubted myself. Becoming a mother in graduate school meant more exhaustion, longer nights, and earlier mornings, but then again it gave me a heightened level of determination. With the enduring love and support of my husband, I was able to overcome any obstacle that graduate school offered. I can only hope that I am able to set forth the same example for my children as my parents, Earl and Lisa Clardy, have done for me. Whether it is financial or emotional support, my parents have always stood by my side and helped ensure my success along the way. Therefore, I also dedicate this work to my parents because without their love, guidance, and support this journey would not have been possible. God has truly blessed me.

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CHAPTER I

INTRODUCTION

Weeds are the target of extensive research because of their impact on horticulture and agriculture. The majority of this research is largely directed towards eradication methods. Over the years, international trade has led to a number of both intentional and accidental non-native plant introductions into the United States. Many of these plant introductions have become problematic, especially invasive weed species. Biological invasions by flowering plants have become more significant as a result of increasing human activities that affect species dispersal (through trade and travel) and habitat vulnerability (through changes in disturbance regimes) (Pimentel et al. 2000). Overshadowed by its aesthetic appeal and its monetary value as a biofuels crop, the invasive potential of the genius *Miscanthus* may perhaps become a real threat to our natural environment.

There are mounting concerns surrounding the invasiveness of *Miscanthus*; in particular, *M. sinensis*. While *Miscanthus x giganteus* is considered sterile, *M. sinensis* is highly prolific with rapid growth rates that have resulted in nontarget site invasions (Raghu et al. 2006). Giant miscanthus (*Miscanthus x giganteus*), also known as giant silvergrass, is a warm season perennial grass native to eastern Asia (Wilson 2011). This hybrid is a cross between silver banner grass (*M. sacchariflorus*) and Chinese silvergrass (*M. sinensis*) and belongs in the family Poaceae. These grasses are rhizomatous with C_4

photosynthetic pathways. The genus *Miscanthus* encompasses some of the most robust and attractive ornamental grasses which include more than 20 species and containing over 50 cultivars (Greenlee 1992). Reaching a height in excess of 3.6 m tall, *Miscanthus x giganteus* is by far the largest *Miscanthus* species. Miller et al. (2010) described this attractive grass as having upright-to-arching leaves that are long and slender with white upper mid-veins and several loosely plumed panicles that turn almost silver to pinkish in the fall. According to Greef and Deuter (1993), this massive grass was first observed by Olson in Yokahoma, Japan in 1935 and later put into cultivation by Karl Foester in Denmark.

Paul Meyer of the University of Pennsylvania's Morris Arboretum first introduced evergreen Eulalia (*M. transomorrisonensis*) from Taiwan in 1979 shortly after Kurt Bluemel began commercializing ornamental grasses in the United States (Darke 1994). Due to the aesthetic appeal of these grasses, *Miscanthus* was quickly popularized in the United States and can be found in numerous landscapes across the country. In recent years, *Miscanthus x giganteus* has attracted attention as a promising biofuels crop due to the biomass production that is twice that of the native switchgrass (*Panicum virgatum*) (Khanna et al. 2008) and 2.5 times the amount of ethanol produced per hectare of corn (*Zea mays*) (ScienceDaily 2008). Europe has reported 7 to 27 t ha^{-1} yr^{-1} yields of *Miscanthus x giganteus* in commercial production sites; therefore, fewer hectares of farmland would be dedicated to biofuels crop production (ScienceDaily 2008). High yield combined with other plant characteristics, such as cold temperature tolerance, low fertility requirements, annual harvest, low water needs, and no known insect or pathogen pest makes *Miscanthus x giganteus* a preferred potential biomass source (Scurlock 1999;

Pyter et al. 2007). However, these same characteristics that make it attractive as a biofuels crop, also make it a potentially serious weed.

The most successful weeds are not always classified as noxious. Noxiousness implies difficulty of extermination and a strong tendency to depress the growth and reproductive output of other plants (Baker 1974). *Miscanthus x giganteus* is capable of high productivity on marginal soils; however, due to its sterility, it receives a low invasive Weed Risk Assessment (WRA) score for the United States. Conversely, *M. sinensis* is highly prolific and capable of producing viable seed in the United States (Meyer and Tchida 1999). Known as a pioneer species in its native range, *M. sinensis* is capable of colonizing and ultimately dominating heavily disturbed volcanic sites (Tsuyuzaki and Hase 2005) and clear-cuts (Ohtsuka et al. 1993).

Countless invasive plants have agronomic or horticultural origins with extended periods of cultivation that lead to their escape, naturalization, dispersal, and negative environmental impacts (Mack 2000). Due to the 2007 Energy Independence and Security Act (EISA), *Miscanthus* is being put into production to help decrease the use of fossil fuels and create renewable sources of energy (US Congress 2007). As the world's population continues to increase, non-renewable natural resources are diminishing due to rapid increases in modernization and industrialization. In 2012, Aloterra Energy and MFA Oil Biomass committed 7,284 ha of marginal land to the production of *Miscanthus* in four different project areas with the hopes of growing these project areas to 20,234 ha (USDA-FSA 2011). Under the Energy Independence and Security Act (EISA), a goal was established to produce over 79 billion liters of advanced biofuels annually by 2022 (Maung and Gustafson 2010).

Implications of Sterility

The impact of introduced species on native species has been well documented. Species that are deliberately introduced account for half of all problematic introductions (Mack and Erneberg 2002). The ability of an organism to achieve evolutionary success needs to be considered according to the quantity of individuals in existence, the extent of their reproduction, the area of the world's surface they occupy, the range of habitats they can enter, and their potential for putting their offspring in a position to further their genetic line through time (Baker 1974). For bioenergy crops, warnings about invasiveness are based primarily on the accepted WRA protocols (Barney and Ditomaso 2008; Cousens 2008; Buddenhagen et al. 2009).

Due to lack of seed production, the WRA protocols rate *Miscanthus x giganteus* in the low category for invasiveness potential (Lewandowski et al. 2000); however, sterile grasses often spread successfully as weeds, giant reed (*Arundo donax*) serving as an extreme example (Raghu et al. 2006). Unfortunately, sterility cannot be certain with any plant species. Although beneficial to the environment, the sterility of *Miscanthus x giganteus* poses limitations for agronomic production. Due to its sterility, rhizome division and in-vitro cultures are the only options for propagation (Clifton-Brown and Lewandowski 2002). Because of better economic seed propagation methods, *M. sinensis* clones appear to be superior to *Miscanthus x giganteus* (Defra 2004) for biofuels crop establishment. The high cost involved in the mass propagation of *Miscanthus x giganteus* has led to the consideration of its more invasive seed propagated parent, *M. sinensis*.

M. sinensis can be used as breeding material given its fertility and rich genetic diversity (Stewart et al. 2009); whereas, only a few genotypes of *Miscanthus x giganteus* are available. In addition, *M. sinensis* is capable of producing biomass yields comparable to that of *Miscanthus x giganteus* (Christian et al. 2005). When evaluating *Miscanthus x giganteus*, *M. sacchariflorus*, wild *M. sinensis*, and bred *M. sinensis* hybrids over three years, a study conducted in Germany determined that yields were highest for *Miscanthus x giganteus* and some newly developed *M. sinensis* hybrids, but biomass qualities were best in the pure *M. sinensis* genotypes (Clifton-Brown and Lewandowski 2002). Research conducted in Denmark found *Miscanthus x giganteus* has a lower combustion quality in contrast to *M. sinensis* genotypes (Jorgensen 1997). In comparison to *Miscanthus x giganteus*, European research found that *M. sinensis* adapted well to a wider range of climatic zones and it was determined that *Miscanthus x giganteus* fared poorly over winter in the first year after planting (Pude 1998; Schwarz et al. 1995). Additionally, using a single clone holds a considerable risk of attack from diseases and pests (Clifton-Brown and Lewandowski 2002).

Limited research has been conducted on the control and eradication of *Miscanthus* species, especially *Miscanthus x giganteus*. Because of the lack of research, assessing the control methods of grasses with similar growth habits such as cogongrass (*Imperata cylindrica*) or johnsongrass (*Sorghum halepense*) might provide some guidance. Baker (1974) compiled a list of twelve ideal weed characteristics, several of which are displayed by *Miscanthus*, cogongrass, and johnsongrass. These characteristics include: 1) germination requirements are fulfilled in many environments; 2) discontinuous germination and great longevity of seed; 3) rapid growth; 4) continuous seed production for as long as growing conditions allow; 5) self-compatible, but not completely autogamous or apomictic; 6) seed is wind dispersed; 7) high seed output in good environmental conditions; 8) produces some seed in various environmental conditions; 9) has adaptations for long and short distance dispersal; 10) if a perennial, has strong vegetative reproduction or regeneration from fragments; 11) if a perennial, has brittleness, so not easily pulled from the ground; 12) has ability to compete interspecifically by special means (rosette, choking growth, allelochemicals). Understanding characteristics such as these help researchers combat the ever growing need to improve control methods.

Genotypes of *M. sinensis* are able to withstand a number of stressful conditions, including cold temperatures, low soil pH and fertility soils, repeated burnings, and heavy metals (Stewart et al. 2009) and it has been known to tolerate shade in the United States (Meyer 2003; Horton et al. 2010). Before wide scale production in the biofuels industry is considered, characteristics such as these warrant further more comprehensive evaluations into the invasive potential of *Miscanthus* varieties that are being considered for cultivation.

Various *Miscanthus* species have already been listed as state noxious weeds in Connecticut, Hawaii, and Massachusetts. Wind dispersed seeds have allowed volunteered plants to escape from their planted sites. Wind dispersal has been associated with invasion success in numerous plant species (Gasso et al. 2009; Lloret et al. 2005). Environmentalists and some scientists are apprehensive about the prolonged sterility of *Miscanthus* cultivars. The possibility of viable seed being produced is a concern once *Miscanthus* has had time to adapt to the United States environment. As unrelated cultivars become established near these various *Miscanthus* species, cross pollination could potentially occur that results in viable seed. This type of unwanted cross pollination has already occurred with the noxious and invasive weed cogongrass. The triploid sterility of *Miscanthus x giganteus* could break down during rare recombination events, producing fertile allopolyploid and diploid gamets (Ramsy and Schemske 1998). Such circumstances are considered a rarity; however, fertile seeds of *Miscanthus x giganteus* have been reported (Linde-Laursen 1993). On the other hand, twenty years of trial research in the European Union found no evidence of invasiveness being displayed in *Miscanthus x giganteus* (Long et al. 2007). But, the question remains, is twenty years an adequate time span to evaluate the evolution and adaptation of a plant in a new environment?

Despite the fact that *Miscanthus x giganteus* is a sterile triploid, its invasive potential cannot be eliminated because of its aggressive vegetative rhizomes. Cordgrass (*Spartina spp*.) is a triploid hybrid that produces viable seed (Raghu and Davis 2007) and although giant reed is a triploid, it has become a major weed issue in California waterways due to rhizome fragmentation (Mack 2008). Comparable to *Miscanthus*, giant reed has rapid growth rates and the capability to recover quickly after fires; therefore, allowing it to form extensive climax stands and outcompete native vegetation in California (Rieger and Kreager 1989; Bell 1994). The number of chromosomes in a plant is not always indicative of its invasive potential. In case of a potential outbreak, several methods of control for *Miscanthus x giganteus* have been proposed, including glyphosate applications (Harvey and Hutchens 1995), plowing (Powlson et al. 2005), and repeated glyphosate or fluaziflop-p applications followed by fall tillage (Speller 2003). To date, there has been very limited published research on the control of *Miscanthus x giganteus*.

Comparison of Similar Grasses and Control Methods

Cogongrass, johnsongrass, quackgrass (*Elymus repens*), and giant reed are all rhizomatous perennial grasses that are known for their rapid growth and unwanted distribution across the United States. Just like *Miscanthus*, cogongrass is a native of Asia which was accidentally and intentionally brought to the United States. In 1912, cogongrass entered the state of Alabama through contaminated packing material and was later intentionally planted for forage (Jose et al. 2002). Once cogongrass was considered unpalatable as a forage, Soil Conservation Services used the grass for soil stabilization and unfortunately, this helped disperse cogongrass seed and rhizomes throughout the Southeast (Jose et al. 2002). Due to the invasiveness, competitiveness, and difficulty to control, this perennial rhizomatous grass is ranked as the seventh worst weed species in the world (Holm et al. 1977; Dozier et al. 1998).

Some species of *Miscanthus* have demonstrated allelopathy, which is defined as a damaging effect from a donor plant to the recipient plant by chemical released into the soil (Rice 1984). In Taiwan, there is a unique pattern of herb exclusion by the *Miscanthus* stands, which occupy a large area of hillside. Chou and Chung (1974) found seven phytotoxic substances in the aqueous leaf solution and soils extract of *M. floridulus* that exhibited significant inhibition on the growth of lettuce, used to test allelopathy. This allelopathic phenomenon was also noted in *M. transmorrisonensis* (Chou and Lee 1991). Allelopathic studies have not been conducted on other *Miscanthus* species; however, it is believed that the other *Miscanthus* species could be capable of similar defense mechanisms. This same chemical form of defense has been found in cogongrass (Eussen 1979; Casini et al. 1998; Cerdeira et al. 2012; Koger and Bryson 2004); therefore, enhancing its ability to produce invasive stands while inhibiting the growth of other vegetation.

The success of an exotic invading species depends partly on its capability to multiply and establish rapidly in new habitats. Just like cogongrass, most *Miscanthus* species are capable of both sexual and asexual reproduction. Both plants produce wind dispersed seed. Cogongrass was reported to have low seed germination rates (Shilling et al. 1997) and short viability (Dozier et al. 1998); however, Burnell (2005) later reported cogongrass to have high germination rates. Matumura and Yukimura (1975) found good germination of *M. sinensis* and *M. sacchariflorus*, but noted insufficient seed set for forage production. Seed viability and germination test in southern Florida determined that *M. sinensis* has 77% seed viability and a germination rate of 90% for the viable seed (Wilson and Knox 2006). According to the Early Detection and Distribution Mapping System (EDDMaps) (2016), *M. sinensis* has escaped cultivation in 26 states (CA, CO, MA, RI, CT, DE, MD, NY, NJ, PA, MI, MO, IL, IN, OH, WV, VA, KY, TN, NC, SC, MS, AL, GA, LA, and FL) and is listed as invasive in Illinois, Kentucky, Tennessee, Alabama, Georgia, South Carolina, New Hampshire, and Connecticut.

According to a survey conducted in Japan, *M. sinensis* was found to be the most troublesome weed by the employees of the National Railways' maintenance depots (Ito et al. 1982). Also, *M. sinensis* was considered a top invader of rice (*Oryza sativa L.*) when cultivation was halted (Hakoyama et al. 1977). *Miscanthus* has been a difficult-to-control weed in Japan for years. Since *Miscanthus* has generally been cultivated for ornamental or agronomic purposes, only a limited amount of research has been conducted on eradication methods using physical, biological, or chemical control.

Due to physiological similarities and the lack of existing research, it is beneficial to compare cogongrass control since it may have similar impacts on *Miscanthus* control. Cogongrass, just like *Miscanthus* species, spreads mainly by way of rhizomes and seeds (Dozier et al. 1998). Rhizomes are its primary mechanism for local regeneration and spread (Dozier et al. 1998); therefore, manual and mechanical practices such as hand hoeing and tillage are useful, especially in third world countries where labor costs are low. *M. sinensis* and *M. sacchariflorus* form different types of rhizomes. While *M. sinensis* produces a tuft forming rhizomes with a thin stem, *M. sacchariflorus* has a broad creeping and thick stemmed rhizome (Lewandowski et al. 2003). *Miscanthus x giganteus*, a hybrid of the two, forms a rhizome intermediate between these types (Lewandowski et al. 2003). Both cogongrass and *Miscanthus* grow vigorously (Koger and Bryson 2004; Lewandowski et al. 2003) and have extensive fibrous root systems (Koger and Bryson 2004; Arduini et al. 2006).

For mechanical cogongrass control, Haigh (1951) suggested digging to a depth of 45 cm to remove the rhizomes to achieve control; however, this method of control is only practical when dealing with small patches in open sites. Research has documented cogongrass rhizomes may grow as deep as 120 cm (Holm et al, 1977; Gaffney 1996). *Miscanthus* rhizomes occur mainly in the top 10 cm of soil (Harvey and Hutchens 1995); therefore, mechanical and manual control such as tillage and hand hoeing should be more effective compared to cogongrass, but soil disturbance and potential sedimentation from soil runoff into surface water could be problematic with mechanical control. Some of the most efficient management practices consist of more than one control method, such as burning followed by herbicide application, and then establish cover crops, or mowing combined with tillage. Cogongrass is dependent on fire and relies on burning for dispersal and survival; therefore, burning and mowing favor cogongrass spread from seed by removing ground litter that isolates seed from contact with mineral soil (King and Grace 2000). In Asia, *M. sinensis* grasslands, just like cogongrass, depend on burning as a means to maintain the flora composition of the fire dependent ecosystem which diminishes the litter layer to allow for nutrient cycling (Iizumi 1976; Yamamoto et al. 2002). Fire is a known stimulus of annual and perennial grass growth (DiTomaso et al. 1999; Sheley et al. 1999); therefore, the use of cover crops, herbicides, or tillage, in addition to burning, may help reduce above ground biomass with invasive rhizomatous plant species.

Cogongrass growth can be repressed by restricting the amount of available solar radiation with the use of herbaceous cover crops and possibly through allelopathic interactions (Eussen 1981). *Miscanthus*, just like cogongrass, is known for low-nutrient requirements (Lewandowski et al. 2003). Akobundu et al. (2000) believed that growing leguminous cover-crops would improve the nutrient status of the soil; thereby, enhancing crop growth and competition against cogongrass. Cogongrass is a weak competitor in fertile soils and is sensitive to shading (Ivens 1980). Comparatively, *M. sinensis* is capable of maintaining high photosynthetic rates and positive carbon gains under shaded conditions (Horton et al. 2010). While useful, tillage and cover-crops alone may be insufficient for control because *M. sinensis* has a strong ability to endure both favorable and stressful environmental conditions like shade and marginal land.

Knapp (1985) and Blair (1997) found that C_4 grasses, such as those found in North American tall grass prairies, exhibit higher aboveground productivity when burned annually compared with grasses that were not burned. Willard et al. (1990) reported that cogongrass control was most effective when herbicide treatments were applied after mowing or burning. After burning, rhizomes are forced to utilize stored starch to produce new aboveground shoots. In order to deplete the rhizomes of invasive perennial grasses, burnings and herbicide application timing is crucial for weed control. For noxious weeds, burns should be conducted before viable seed production has occurred and herbicide application timings are dependent upon the type of herbicide used. Currently, no research is available on proper application timings for *Miscanthus*; however, a general strategy often used in the management of perennial weeds is the application of herbicides at growth stages when maximum basipetal transport of carbohydrates occurs (Banks et al. 1977; Bixler et al. 1991; Edenfield et al. 1998; Mitra and Bhowmik 1999; Orfanedes and Wax 1991; Shaw and Mack 1991; Shaw et al. 1990). In late fall, plants begin sending carbohydrates into the roots and rhizomes for storage; therefore, this is considered an excellent time for most systemic herbicide applications to maximize herbicide movement in the plant. During this time, carbohydrates along with the herbicides are translocated into the rhizome.

Currently, glyphosate and imazapyr are the most effective treatments recommended for cogongrass control (Dozier et al. 1998). While researching postemergence herbicides tolerance in *Miscanthus x giganteus* and *M. sinensis*, Everman et al. (2011) found glyphosate caused an estimated 54% injury on *Miscanthus x giganteus* when applied at 0.84 kg ae ha^{-1}; however, no treatments, including glyphosate caused greater than 5% injury on *M. sinensis*. In field and greenhouse experiments, Anderson et al. (2011) investigated combinations of glyphosate and tillage on mature stands of

Miscanthus x giganteus and determined that eradication would require multiyear applications. Tanner et al. (1992) reported that glyphosate as a 2% solution appeared to control burned cogongrass satisfactorily for at least two years. With the incorporation of tillage, perhaps a similar treatment would yield good results for *Miscanthus* control.

Similar introductions of plant species for agronomic benefit have proven to be problematic following cultivation and distribution from one location to the next in the United States. Kudzu (*Pueraria lobata*) and johnsongrass were introduced into the United States in the 1800s and widely promoted for forage. Due to the rooting structure, perennial habit, and extraordinary growth rate of kudzu, it quickly escaped cultivation and began dominating the southeastern United States (Barney and Ditomaso 2008). Johnsongrass posed a similar dilemma in the southeast with its aggressive perennial growth on arable land, waste lands, roadsides, irrigated fields, and field borders (Holm et al. 1991).

Johnsongrass was introduced into the United States from the Ottoman Empire in the early part of the 19th century (Ball 1902; McWhorter 1971) at a time of extensive need for forage due to use of draft animals for transportation and labor. As farming and transportation were mechanized, the demand for johnsongrass forage decreased, but strategies to eliminate johnsongrass from fields where it had been grown for forage when other crops were planted in those sites did not exist. Like *Miscanthus*, johnsongrass is capable of reproduction through sexual and vegetative means (Warwick and Black 1983). This plant is a prolific seed producer that relies on the wind, water, and animals for seed dispersal. It has been estimated that the rhizomes of an individual plant are capable of producing 5,000 nodes in one growing season (Anderson et al. 1960; McWhorter 1961).

Due to its prolific nature, johnsongrass is also considered one of the world's ten worst weeds (Holm et al. 1977). Control methods for johnsongrass have been intensely researched through the years. Many of the herbicides used for johnsongrass control were the focus of this thesis research.

Johnsongrass is capable of tolerating a wide range of environmental conditions, except deep shade. Quite often, it grows in pastures, fields, prairies, roadsides, and waste places. Crops such as soybeans (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), grain sorghum (*Sorghum bicolor* L.), and corn (*Zea mays* L.) are susceptible to johnsongrass invasions (Holm et al. 1977). With stems reaching 1.8 m tall and leaf blades up to 2.5 cm wide, johnsongrass is a robust plant very similar to *M. sinensis*. Suppressing rhizome regrowth and development is essential to the control of such an aggressive plant. Currently, there are limited biological, mechanical, and physical control methods in use; however, there are a number of chemical control methods that have been evaluated and determined to be effective.

In the United States, a common practice used to control johnsongrass is intensive livestock grazing. Three to four years of heavy grazing can significantly reduce plant populations (McWhorter 1989). Livestock grazing is not a common practice used for *Miscanthus* control in the United States; however, Meyer (2008) reported it is used as a management strategy in Japan. In Asia, *M. sinensis* is a vital forage resource in native forest and grasslands because of its high palatability to domestic herbivores (Itow 1962). While observing *M. sinensis* under livestock grazing, Hirata et al. (2007) determined that the size (height and basal area), shoot number of tufts, and number of live leaves per shoot had significantly declined during two years. This study demonstrated that *M.*

sinensis has no tolerance for livestock grazing; furthermore, grazing might have value as a biological control method.

Mowing is a common practice for johnsongrass suppression, but it does not eradicate this plant. A primary source of reserve energy stored in the rhizomes of forage grasses and johnsongrass is total nonstructural carbohydrates (TNCs); furthermore, regrowth potential after mowing or clipping has been correlated to rhizome TNC content (Smith 1981). Johnson, Li, and Wait (2003) evaluated TNC levels of johnsongrass after the application of postemergence herbicides and determined that quizalofop and glyphosate caused a 64 and 61% reduction in rhizome TNC, respectively. Compared to the nontreated plants in this greenhouse study, both quizalofop and glyphosate provided greater than 95% biomass reduction in johnsongrass (Johnson, Li, and Wait 2003).

Miller et al. (2010) listed five management strategies for *M. sinensis* including: 1) Do not plant. Remove prior plantings, and control seedlings and sprouts. Dispose of plants and seed heads by burning; 2) treat when new plants are young to prevent seed formation; 3) minimize disturbance within miles of where fertile plants occur, and anticipate wider occupation if plants are present or adjacent before disturbance; 4) do not mow when seed heads are present; 5) burning treatments are suspected of having minimal effect, and dormant standing infestations in winter are highly flammable and pose a fire hazard. For control strategies, a combination of imazapyr (Arsenal AC* as a 5% by volume solution) plus glyphosate herbicide (4% by volume solution of a 41% active ingredient formulation) is recommended as a fall application (Miller et al. 2010). Applications can be repeated when new growth reaches 61 cm in height. Although research has indicated Arsenal controls *Miscanthus*, residual activity from this herbicide may prevent the production of other many annual crops for at least two growing seasons following application.

Invasive species threaten our agricultural productivity, native biodiversity, and ecosystem functioning, with estimated annual impacts that amount to millions of dollars (Pimentel et al. 2000). In order to eradicate current and future outbreaks of *Miscanthus*, further research is necessary to develop more efficient control strategies. Certainly, independence from foreign oil is a major priority of the United States; however, protecting our natural resources and environment from the spread of invasive nonnative plants should be of equal concern. In the event that *Miscanthus* is not the promising biofuels crop as it has been portrayed, there must be a means for producers to quickly convert fields from *Miscanthus* to something more economically beneficial. Tull (1762) wrote the weeds most difficult to kill were those that reproduced not only by seeds, but also by roots. Therefore, the purpose of this study is to determine an effective chemical control method for *Miscanthus*.

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CHAPTER II

RHIZOME INITIATION IN SEEDLING *MISCANTHUS* AND EFFECTS OF MOWING ON RHIZOME PRODUCTION

Abstract

In 2014 and 2015, greenhouse experiments were conducted at the RR Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS with two objectives: 1) evaluate the time interval required for *Miscanthus* seedlings to produce rhizomes after germination and 2) determine if seedling *Miscanthus* terminal removal (mowing) stimulates rhizome production. Experiments were repeated in time with each planting in late winter. A variety of *Miscanthus x giganteus* seed, ‘Powercane’ $^{\mathrm{TM}}$, was germinated in one L size pots filled with Miracle Grow potting mix, then transplanted into 61 cm x 5 cm x 30 cm plexiglass sided rhizotrons after reaching an average height of 35 cm. Plexiglass sides were covered with foam board insulation to exclude sunlight. Ten weeks after germination (WAG), half of the seedlings were cut to a stubble height of 10 cm to simulate mowing. Plants were monitored weekly for rhizome initiation. At the conclusion of each experiment, culm height, number of lateral shoots, number of rhizomes, rhizome fresh weight, rhizome dry weight, aboveground fresh weight, and aboveground dry weight were recorded. Rhizomes were visible on uncut plants 15 and 13 weeks after germination, respectively, in 2014 and 2015. *Miscanthus* that had the terminal removed by cutting produced visible rhizomes at 19 and 13 WAG, respectively, in 2014 and 2015. Analysis of the number of shoots and rhizomes, shoot height, total aboveground biomass, and rhizome biomass, revealed a significant difference ($P < 0.05$) in these biomass measurements between uncut and cut plants. A 66 and 27% decrease in the number of rhizomes produced was noted in *Miscanthus* plants with terminals removed compared to plants with intact terminals, respectively, in 2014 and 2015. However, plants with terminals removed produced 26 and 12% more aboveground shoots than their counterparts, respectively, in 2014 and 2015. This corresponded to a 45 and 1% increase in aboveground biomass. Compared to their counterparts, an 11% increase in 2014 and 7% decrease in 2015 was noted in the height of plants that had terminals removed. Terminal removal in seedling *Miscanthus* appeared to hinder rhizome development, but stimulate lateral shoot numbers and overall aboveground biomass. The implications for control of escaped seedling *Miscanthus* is although mowing seedlings will retard rhizome development, this practice will stimulate lateral shoot development and increase aboveground biomass. Mowing has the potential to result in a thicker and denser *Miscanthus* stand which may be more difficult to eradicate with chemical treatments.

Nomenclature: *Miscanthus*, *M. x giganteus*, PowerCane.

Abbreviations: WAG, weeks after germination.

Keywords: Biofuel crop, rhizome, rhizotron, terminal, biomass.

Introduction

Miscanthus x giganteus is capable of producing twice the biomass of switchgrass (*Panicum virgatum*) (Khanna et al. 2008) and 2.5 times the volume of ethanol per hectare compared to corn (*Zea mays*) (ScienceDaily 2008). Because of this, in the biofuels industry, *Miscanthus* hybrids have quickly become one of the main potential crops of interest and large scale production operations have begun. On commercial production sites in Europe, growers reported 7.4 to 27.2 t ha^{-1} yr^{-1} yields of *Miscanthus x giganteus* (ScienceDaily 2008). Landowners are being enticed to grow *Miscanthus* as a biofuel crop in place of other crops such as corn, cotton, bermudagrass, alfalfa, and soybeans. With assistance from Aloterra Energy and MFA Oil Biomass, more than 200 farming families in Arkansas, Missouri, Ohio, and Pennsylvania have dedicated 7,300 hectares of marginal land to the production of *Miscanthus* (Gibson 2011).

Learning from History

In 2003, the Planning Commission of India set a goal to have 30% biodiesel blended into petroleum diesel fuel by 2020 (Kant 2011). To accomplish this goal, India focused on *Jatropha curcas* as a renewable energy source partially because, like *Miscanthus*, it is drought tolerant and can survive on marginal lands (Kant 2011). In India, farmers were encouraged to participate in a wide scale planting program of unprecedented proportions. Soon after, China, Tanzania, and Africa followed suit and by 2008, *Jatropha curcas* was planted over an estimated 900,000 ha globally (Kant 2011). By the end of 2015, *Jatropha curcas* was predicted to cover 12.8 million ha worldwide (Kant 2011).

Jatropha curcas produces seed that contains a viscous oil which can be used to produce soap, cosmetics, or diesel/kerosene substitute (Openshaw 2000). Many promoted *Jatropha curcas* as a solution to global warming and the greenhouse effect. Unfortunately, those advertisements were quickly put to rest because of technical and economic reasons. Seed production fell far short of expectations in India (Kant 2011). A research experiment in Tanzania found the net present value of a five-year investment in *Jatropha curcas* was a loss of $65 US dollars per ha (Kant 2011). A vast number of farms were involved in the *Jatropha curcas* planting program, which turned out to be an epic failure. *Jatropha curcas*, like *Miscanthus*, has a long history as a problematic weed. Plantings of *Miscanthus* are prohibited in Australia (Low and Booth 2007). *Miscanthus* could become the next *Jatropha curcas*. Therefore, to protect current and future United States farmers, weed scientist must examine every biological aspect of this plant. As Niccolo Machiavelli (1469-1527) once said, "Whoever wishes to foresee the future must consult the past; for human events ever resemble those of preceding times."

Clonal Plants

On a smaller scale, the history of kudzu (*Pueraria lobata*) in the United States parallels *Jatropha curcas* in India. In the 1870s, kudzu was introduced from Japan (Miles and Gross 1939; McKee and Stephens 1943). Kudzu was promoted throughout the southern United States decades later as a forage and green manure crop and for erosion control (McKee and Stephens 1943; Miles and Gross 1939; O'Brien and Skelton 1946; Semple et al. 1934). As a perennial plant, kudzu has a tuberous root system that can reach a depth of nearly 4 m and weigh as much as 136 kg (Everest et al. 1991). In the 1930's, 485,630 ha of kudzu was planted in the United States for erosion control (Britton et al. 2002). Landowners in the south were paid $20 per ha^{-1} to plant kudzu under a subsidized program developed by the Soil Erosion Service (Britton et al. 2002). During this time period, kudzu festivals were held and kudzu queens were crowned.

Soon kudzu engulfed forested lands and completely replaced existing vegetation. Kudzu control costs exceeded $494 ha^{-1} y^{-1} for five years; therefore, timber production was not economically feasible in kudzu infested property (Britton et al. 2002). Direct losses from kudzu are estimated at $500 million annually (Quimby et al. 2003). By 1998, kudzu was listed by the United States Congress as a Federal Noxious Weed (Britton et al. 2002). Everest et al. (1991) estimated 2.8 million ha of land in the southeastern United States were infested with kudzu with the heaviest infestations occurring in Alabama, Georgia, and Mississippi.

Many introduced plants become weedy in a new environment. Of the 25 weeds listed as most harmful to United States agriculture in the 19th century, 19 were introduced from other countries (Anonymous 1898). Plants have been introduced for an array of reasons ranging from forage to medicinal purposes in addition to accidental introductions as contaminants in or on other products. But the potential for these plants to become weeds is often ignored, overlooked, or not known. Unfortunately, we fail to draw parallels between past and present introductions of potentially invasive weeds. Classic examples of plants that were intentionally introduced and became problematic in the United States include: johnsongrass (*Sorghum halepense*), quack-grass (*Elymus repens*), cogongrass (*Imperata cylindrica*), kudzu, yellow nutsedge (*Cyperus esculentus*), large crabgrass (*Digitaria sanguinalis*) and far too many others to list. *Miscanthus* shares many similar traits that caused the prolific spread of these plants as weeds.

Rhizome growth and development in weeds such as quackgrass can be rapid. During the summer months, quackgrass rhizomes are capable of growing up to 25 cm weekly (Hakansson 1967). Hakansson (1967) found the diameter of a single quackgrass rhizome spread was 3.3 m; 14 rhizomes from the original plant had grown to a total length of 135 m; 206 aerial shoots were produced by the system; and 232 additional growing point were found on rhizomes. Quackgrass rhizomes have been used for medicinal purposes for many years (Henkel 1904). During the late 19th and early 20th century, 113,400 kg of quackgrass rhizomes were imported into the United States annually from Europe as a remedy for kidney and bladder discomfort (Henkel 1904). Synthetic pharmaceuticals eliminated the need for medicine derived from quackgrass rhizomes, but unfortunately, quackgrass was widely established in the United States. It is reported to be a weed in 32 crops in more than 40 countries (Holm et al. 1991).

Jethro Tull (1762) wrote plants that reproduced by roots in addition to seeds were the most difficult to control. Control of plants that produce rhizomes, prior to rhizome initiation, should be more effective than control attempts after rhizome production. Johnsongrass can produce rhizomes 28 to 56 days after seed germination (Keeley and Thullen 1981) and cogongrass seedlings are capable of initiating rhizome production within 30 to 40 days (Patterson et al. 1981). Oyer et al. (1959) noted that the initiation of rhizomes by johnsongrass seedlings occurred when plants were in the seven-leaf stage (about 50 days after planting). This leaves a narrow application period to implement control measures while the plant is still an annual before rhizome production. Anderson et al. (1960) determined that a johnsongrass plant is capable of producing 5,200 internodes in as little as 4.5 months. Johnsongrass rhizomes can grow to length of 2.7 m and produce up to 33,600 kg/ha^{-1} rhizomes annually (Stamper 1957). Distribution of rhizomes on perennial grasses is partially dependent upon soil texture. Johnsongrass rhizomes occur mostly in the top 20 cm of soil; however, these structures can reach depths of 50 cm in cultivated soil (Rayburn 1996). Cogongrass rhizomes typically occur in the upper 15 cm of fine textured soils, but can extend to depths of 120 cm (Gaffney

1997; Holm et al. 1977). By comparison, *Miscanthus* and quack-grass rhizomes are reported mainly to occur in the top 10 cm of soil (Harvey and Hutchens 1995; Hakansson 1967).

As rhizomes develop deeper into the soil profile, they become increasingly harder to control. Very little is known about the vast root system of *Miscanthus*. However, the massive amount of aboveground biomass suggest a certain level of comparability with johnsongrass and cogongrass. While johnsongrass can produce 5 to 12 t of aboveground biomass ha^{-1} y^{-1}, *Miscanthus x giganteus* can produce 7 to 27 t ha^{-1} y^{-1} (Ball et al. 2007; ScienceDaily 2008). If *Miscanthus* has the same low shoot-to-root ratio as cogongrass, then control would be quite the undertaking for landowners that desire to eradicate *Miscanthus* to use land for other purposes, especially crop production.

Clonal plant species, such as *Miscanthus sinensis*, can produce offspring through clonal propagation and by sexual reproduction of seed (Piquot et al. 1998). Perennial seedlings cannot reproduce asexually until they have developed a vegetative reproductive organ, such as a rhizome (Anderson 1999). These vegetative structures make perennial plants more difficult to control as each node on the rhizome is capable of sprouting a stem. Johnsongrass, quackgrass, kudzu, and cogongrass are examples of clonal plants. For clonal plant species, seed production is the primary means of long distance dispersal while vegetative propagation mostly contributes to local population expansion (Stebbins 1950). Controlling perennial grasses before rhizome production is initiated is both less strenuous and costly for landowners.

Implications of Dispersal and Effects of Mowing Similar Plants

Currently, data have not been published on the number of seeds *Miscanthus* plants can produce. However, it has been reported that cogongrass and johnsongrass can produce 3,000 and 80,000, respectively, seeds per plant per year (Hartzler and Chappell 1981; Holm et al. 1991). While hybrids such as *Miscanthus x giganteus* are considered sterile, other species such as *Miscanthus sinensis* are highly prolific producers of wind dispersed seed. There is some concern about the prolonged sterility of *Miscanthus* cultivars because of potential cross pollination with an unrelated seed fertile cultivars. While many may discount the probability of such an event, cogongrass in North America was considered sterile for many years (Cseke and Talley 2012).

In 1912, cogongrass was brought into Alabama through contaminated packing material and was later intentionally planted as a potential warm season perennial forage (Jose et al. 2002). Although it was not a desirable forage, Soil Conservation Services encouraged planting cogongrass for soil stabilization, which unfortunately, helped disperse populations throughout the southeast (Jose et al. 2002). In 2005, it was estimated that cogongrass infests between 200,000 to 405,000 ha in Alabama, Mississippi, and the Florida panhandle (Faircloth et al. 2005).

A cultivated variety of cogongrass, ‘Red Baron’, originally thought to be sterile and widely sold as an ornamental plant has been found to revert to an aggressive, green form, and even in its red form, to produce fertile seed and new seedlings (Bryson et al. 2003). Taxonomically, as well as morphologically and genetically, Brazilian satintail *(Imperata brasiliensis*) is nearly identical to cogongrass (Bryson et al. 2010). Where they co-occur, *Imperata cylindrica* and *Imperata brasiliensis* readily hybridize and produce fertile offspring (Masterson 2007). Hybridization has been known to facilitate invasion by other plant species (Ellstrand and Schierenbeck 2000).

Another similarity between cogongrass and *Miscanthus* is the production of wind dispersed seed. Attempts have been made to measure dispersal distances of seeds spread by wind. Yager (2007) measured greater dispersion of cogongrass spikelets in longleaf pine: bluestem understory compared to longleaf pine: shrub understory forests. However, she did not report wind speed when the dispersion was measured. On 28 April, 2014, an EF-4 tornado cut a path less than 3.2 km from a *Miscanthus* biomass planting near Louisville, MS. Approximately 48.3 km away from Louisville, debris, including a door from a house, was discovered on the campus of Mississippi State University (Mersereau 2014). At one point, this particular tornado formed a debris cloud that measured 4.8 km across at 1.5 km above ground level (Mersereau 2014). Wind velocity of that magnitude has the capability to move wind dispersed seed significant distances away from production sites and could spread propagules close to populations of *Miscanthus* planted for ornamental settings that could ultimately result in hybridization. Weed seed can be easily dispersed not only by humans, but by Mother Nature as well.

Perennial weeds are usually mowed for one of three reasons: 1) inhibit seed production, 2) to starve underground plant parts, or 3) aesthetics (Anderson 1999). Anderson (1999) stated, “To be effective, mowing must be done before viable seed are formed, and frequent mowings during the growing season may be required over several years to deplete the stored food reserves.” Aldous (1935) reported that repeated mowing when carbohydrates in buckbrush or sumac roots was at the lowest level resulted in eradication of these two shrubs in Kansas pastures. Burnell et al. (2003) demonstrated that frequent mowing of cogongrass from March to October reduced the number of plants per unit area by 74%; however, cogongrass resprouted after two consecutive seasons of treatment. After five years of weekly mowing to bareground with a string trimmer, stem density and rhizome biomass was reduced 86 and 70%, respectively. Although impractical, exceedingly frequent mowing over five years has shown positive cogongrass rhizome control (Burnell et al. 2003).

Occasional mowing stimulates populations in the perennial weed colonies due to newly emerged stems following the release of dormancy in buds previously held in check by intact aerial shoots (Anderson 1999). Mowing irregularly, at too high a height, or both might increase weed populations; on the other hand, short mowing injures and may weaken desirable vegetation. When looking at mowing as a method to reduce competitive interference between alfalfa (*Medicago sativa*) and legumes, Chamblee (1975) determined that alfalfa density typically increased as cutting intervals were shortened and cutting height lowered. Therefore, mowing alone may not effectively eliminate perennial weed infestations and may actually exacerbate the problem.

Turgeon (1996) stated, mowing within tolerance ranges caused both physiological and morphological changes in turfgrasses, such as stimulated aerial shoot growth, increased shoot density and smaller shoot size, decreased root and rhizome growth, decreased synthesis and storage of carbohydrates, and increased plant succulence. Weinmann and Goldsmith (1948) determined that clipping bermudagrass (*Cynodon dactylon*) had little effect on carbohydrate reserves, unless plants were mowed extremely short. Clipping bermudagrass removes apexes of shoots which stimulates lateral stem development to produce a dense prostrate stand with the capacity to maintain a high photosynthetic rate and therefore, maintain a high level of reserve carbohydrates under frequent mowing (Youngner and McKell 1972). According to Youngner and McKell (1972), cutting stem apexes helps stimulate tillering by removing the major source of auxin which inhibits lateral bud development. Inactive lateral buds are then free to develop (Leopold 1949).

Robertson (1933) evaluated the effects of frequent clipping on the development of certain grass seedlings: blue gramagrass (*Bouteloua gracilis),* Hungarian bromegrass *(Bromus inermis),* sudangrass *(Holcus sorghum sudanensis),* junegrass *(Koeleria cristata),* bluegrass *(Poa pratensis), and* needlegrass *(Stipa spartea)*. He determined that growth of one-half of these perennial grasses was stimulated by clipping and elongation of tops resulted. However, top growth of the other one-half was inhibited by clipping. Clipped Hungarian bromegrass and junegrass, which were clipped four times over the duration of the experiment, shoots elongated 10 and 4 cm more than the control plants, respectively, although neither produced tillers. Also in his experiment, needlegrass height increased 30% after four clippings, but this species also failed to produce tillers. The unclipped blue gramagrass and sudangrass produced 10 and 7 times as many tillers and was 16 and 7 times as tall as the plants that had been clipped 4 times, respectively. Bluegrass plants that had been clipped four times also failed to produce tillers and the control plants were found to weigh 7.6 times more than that of clipped plants. Overall, leaf width, number of leaves, and number of tillers were reduced by clipping in this experiment. Past studies have shown that frequent removal of above ground vegetation limits root development (Graber 1931). Largely, nutrients stored in the rhizomes of grasses are synthesized above ground; therefore, frequently harvesting above ground tissues reduces the plant's capacity to photosynthesize. Cutting mature plants allows for more reserve food and higher yields with decreased winter-killing and a longer life for grass stands (Albert 1927).

Experiments were conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS in 2014 and 2015 with two objectives in mind (1) evaluate the time interval between seedling *Miscanthus* emergence and rhizome production and (2) determine if apex removal, ie cutting seedling *Miscanthus*, stimulates rhizome production.

Material and Methods

Plant Material

'Powercane', a fertile variety of *Miscanthus x giganteus*, was used for these experiments. Seed was provided by Mendel Bioenergy[1] Seeds. To test seed viability, 100 seeds were divided into four plastic petri dishes that contained a piece of filter paper. Water was added to moisten the filter paper. To prevent desiccation, petri dishes were sealed with plastic wrap. Temperature in the growth chamber was set at 32 °C daytime (12 hrs) and 21 °C nighttime (12 hrs). After two weeks, germinated seedlings were counted. Seedlings with a radicle length of 1 mm or greater were counted as germinated to reveal germination rates of 96% and 92%, respectively, in the two germination tests.

Greenhouse Experiment

For these two experiments, 24 wooden rhizotrons, measuring 61 cm long x 5 cm wide x 30 cm tall with plexiglass sides, were constructed of untreated wood to facilitate

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[1] Mendel Bioenergy Seeds, 432 TY TY Omega Rd, Tifton, GA 31793

visual inspection of underground plant development with minimal plant disturbance (Figure 2.1). Foam insulation covered the plexiglass sides of each rhizotron to exclude sunlight. Approximately 11400 cm^3 Miracle-Gro[2] potting mix was used to fill each rhizotron. Greenhouse temperatures could not be regulated with precision; therefore, temperatures ranged from 3 to 34 °C through the duration of the experiments. Supplement light was not provided. For both experiments, five *Miscanthus* seeds were planted into each of 35 1 L pots 20 February, 2014, and 9 March, 2015. The first seedlings germinated 3 March and 15 March, respectively. Plants were thinned by hand removal to one seedling per cup. Once seedlings reached an average height of 35 cm, 24 seedlings were randomly selected and transplanted 1 into each rhizotron. After seedlings were well established and actively growing in rhizotrons by 10 WAG, one-half of the plants were randomly cut with scissors to a culm height of 10 cm to simulate mowing. Based on the treatment, plants were arranged into two groups, cut and uncut. Therefore, experimental design was a randomized complete block with 12 rhizotrons (experimental units) in each of the two groups. For experiment one and two, average culm height prior to cutting was 52 and 110 cm, respectively. Plants were monitored for rhizome initiation weekly. Rhizome development was recorded when rhizomes were visible through the plexiglass sides of the rhizotron. The number of rhizomes, shoot height and number of shoots produced were recorded at the end of each experiment. Plants were watered as needed to maintain soil moisture in the rhizotron. On 11 August, 2014 and 18 August, 2015 studies were terminated when plants reached 23 and 22 WAG, respectively. Culm biomass was harvested with a reciprocating saw at the soil surface level and fresh weights

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[2] Miracle-Gro potting mix, Scotts Miracle-Gro Products Inc., 1411 Scottslawn Road, Marysville, OH 43041

recorded. Below ground biomass was removed from the rhizotrons, cleaned of soil using dry tissue paper, rhizomes separated from roots, counted and weighed. To prevent miscounting rhizomes, new sprouts shorter than 4 mm were not counted. All plant biomass samples were placed in a dryer for four days at 55 °C and then dry weight recorded. A logarithmic transformation (base 10) of rhizome fresh and dry weights, as well as a square root transformation of number of rhizomes were made and the resulting data were analyzed using PROC GLM[3] in SAS 9.3[4] (SAS 2011). Fisher's Protected LSD at the 0.05 level of significance was used to separate treatment means. Data for the two runs of the experiment were not combined for analysis.

Results and Discussion

Analysis of variance revealed significant differences in response variables between experiments in 2014 compared to 2015; therefore, data are presented separately.

Experiment one

The data from multiple response variables revealed the two treatments, cutting terminals and not cutting terminals were significantly different with respect to mean culm height ($P < 0.05$), number of shoots ($P < 0.05$), number of rhizomes ($P < 0.05$), rhizome fresh weight ($P<0.05$), rhizome dry weight ($P < 0.05$), aboveground fresh weight ($P<0.05$), and aboveground dry weight ($P < 0.05$) (Table 2.1).

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[3] The GLM procedure uses the method of least squares to fit general linear models. Among the statistical methods available in PROC GLM are regression, analysis of variance, analysis of covariance, multivariate analysis of variance, and partial correlation.

[4] Statistical Analysis System, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513-2414

Rhizome development in plants with intact terminals were first noted at 15 WAG. Rhizome development was not observed until 19 WAG on plants that had the terminal removed. Therefore, cutting *Miscanthus* seedlings to a stubble height of 10 cm delayed rhizome development four weeks in this experiment. *Miscanthus* seedlings grew to an average height of 52 cm at 10 WAG prior to cutting. The number of rhizomes produced and rhizome dry weight were also significantly impacted by cutting. Plants that were not cut produced an average of 8.9 rhizomes plant^{-1} with a rhizome dry weight of 7.0 g. However, plants that were cut produced only 3.1 rhizomes plant^{-1} with an average dry weight of only 2.0 g. Overall, removing the *Miscanthus* shoot terminal reduced the number of rhizomes produced 66% and total rhizome fresh and dry weight by 63 and 71%, respectively, in experiment one. Frequent removal of above ground vegetation has been shown to limit root development in past studies (Graber 1931). However, *Miscanthus* culm height, number of shoots, aboveground fresh weight and dry weights increased by 11, 26, 48 and 45%, respectively, on plants with the terminal removed in this experiment compared to the average of those plants with terminals not clipped. The response of *Miscanthus* in this greenhouse experiment parallels those reported for turfgrasses. Turgeon (1996) found that mowing turfgrasses within tolerance ranges caused both physiological and morphological changes, such as stimulated aerial shoot growth, increased shoot density, and decreased root and rhizome growth.

Experiment Two

For the multiple response variables, experiment two revealed no significant differences between culm height (P = 0.1051), number of shoots (P = 0.1797), number of rhizomes (P = 0.1358), rhizome dry weight (P = 0.0527), aboveground fresh weight (P =

0.8935), or aboveground dry weight (P = 0.7249) (Table 2.2). Rhizome development in both uncut and cut plants was first noted 13 WAG. Therefore, in the second experiment cutting *Miscanthus* seedlings to a stubble height of 10 cm did not delay rhizome development, nor, significantly alter plant development for any of these response variables. *Miscanthus* seedlings grew to an average culm height of 110 cm at 10 WAG prior to cutting. Plants with the apex removed were significantly different than those not cut with respect to rhizome fresh weight (P = 0.0388) only. Compared to uncut *Miscanthus*, culm height of plants with terminals removed was reduced 7%, the number of rhizomes decreased 27%, rhizome fresh weight reduced by 40%, and aboveground fresh weight lowered by 4%. However, the number of shoots on cut plants increased by 12% in experiment two.

Both experiments one and two exhibited a decreased number of rhizomes and an increased number of shoots on plants with terminals removed. Research conducted by Anderson (1999) found that occasional mowing of perennial weed colonies stimulates newly emerged stems following the release of dormancy in buds previously held in check by auxins. Robertson (1933) found that frequently clipping perennial seedlings of blue gramagrass, Hungarian bromegrass, sudangrass, junegrass, bluegrass, and needlegrass negatively impacted the production of tillers. In addition, one-half of these perennial grasses were stimulated by clipping and elongation of tops resulted; however, the top growth of the other one-half was inhibited by clipping.

Conclusions

Inconsistent results were measured between these two experiments. Removal of the plant apex decreased the mean number of rhizomes produced over the course of both experiments (Figure 2.2 and Figure 2.3), although the difference was not significant in 2015. However, removal of the apex increased the mean number of shoots produced in both experiments while causing aboveground biomass to increase significantly in experiment one, but not in experiment two. Therefore, clipping seedling *Miscanthus* 10 WAG may be insufficient from a control standpoint and may exacerbate the difficulty of control.

Response variables between the two experiments may at least partly differed due to ambient external greenhouse temperatures between the two years these studies were conducted (Table A2.1). Overall, temperatures were warmer in 2015 compared to 2014. *Miscanthus* seed were planted earlier in 2014 compared to 2015, so seed did not germinate as quickly. After terminal removal in 2015, temperatures were consistently warmer compared to 2014. This fluctuation in temperatures could affect a plant with tropical origin. Compared to 2014, uncut plants in 2015 produced 38% more aboveground biomass and 43% more rhizomes. Additionally, seedlings were 53% taller 10 WAG in 2015 compared to 2014. Since *Miscanthus* originated in a tropical environment, seedling *Miscanthus* was probably impacted by the cooler temperatures during 2014. This may explain why rhizomes developed four weeks later on cut seedlings compared to uncut seedlings because no delay of rhizome development was noted between uncut and cut in 2015. In addition, rhizomes developed two weeks sooner in 2015 than 2014. Differences in ambient external greenhouse temperatures between studies might explain these variances and demonstrate the impact temperature plays on *Miscanthus* growth and its ability to adapt to different environments.

Table 2.1 Means of cut and uncut *Miscanthus* seedlings, Plant Science Research Center greenhouse, Mississippi State University for 2014.

Study1	Cut N=12	Uncut N=12	Pr > F	LSD
Culm height	127.2 a	113 b	0.0052	9.5
Number of shoots	45.4 a	33.8 b	0.0039	7.5
Number of Rhizomes	3.1 b	9.0 a	0.0157	4.6
Rhizomes fresh weight	8.2 b	22.0 a	0.0127	10.4
Rhizome dry weight	2.0 b	7.0 a	0.0044	3.3
Aboveground fresh weight	497.4 a	260.4 b	< .0001	28.4
Aboveground dry weight	137.7 a	75.3 b	0.0002	72.1

Means with a different letter across rows/variables are considered significant, according to Fisher's Protected LSD at P = 0.05. Height recorded in cm and weights recorded in g.

Table 2.2 Means for cut and uncut *Miscanthus* seedlings, Plant Science Research Center greenhouse, Mississippi State University for 2015.

Study1	Cut N=12	Uncut N=12	Pr > F	LSD
Culm height	145.1 a	155.8 a	0.1051	13.2
Number of shoots	28.4 a	25.1 a	0.1797	5.0
Number of Rhizomes	11.6 a	15.9 a	0.1358	5.8
Rhizomes fresh weight	19.1 b	31.7 a	0.0388	11.9
Rhizome dry weight	7.4 a	12.5 a	0.0527	5.2
Aboveground fresh weight	400.5 a	417.2 a	0.8935	39.3
Aboveground dry weight	218.3 a	215.7 a	0.7249	97.0

Means with a different letter across rows/variables are considered significant, according to Fisher's Protected LSD at P = 0.05. Height recorded in cm and weights recorded in g.

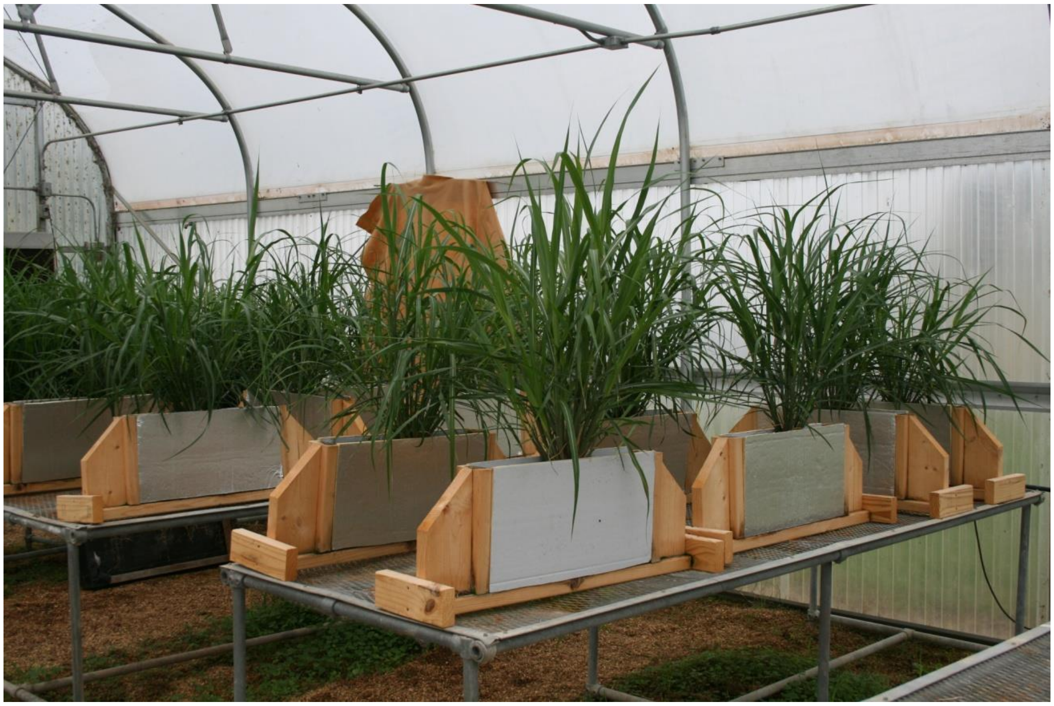

Figure 2.1 Wooden rhizotrons, 61 cm long x 5 cm wide x 30 cm tall used to monitor rhizome development, Plant Science Research Center greenhouse, Mississippi State University in 2014

Figure 2.2 Cut seedling *Miscanthus* 23 weeks after germination, Plant Science Research Center greenhouse, Mississippi State University for 2014

Figure 2.3 Uncut seedling *Miscanthus* 23 weeks after germination, Plant Science Research Center greenhouse, Mississippi State University for 2014

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CHAPTER III

EVALUATION OF HERBICIDE EFFICACY AND APPLICATION TIMING FOR *MISCANTHUS* CONTROL

Abstract

In 2013 and 2014, *Miscanthus* field experiments were conducted near Louisville, MS on the cultivar 'Nagara' and adjacent the MSU Dairy on the cultivar 'Freedom' at Mississippi State, MS with two objectives: (1) determine the efficacy of herbicide treatments and (2) herbicide application timing for *Miscanthus* control. Louisville experiments consisted of 21 herbicide treatments: glyphosate at 2,200, 4,500, 7,300 g ae ha^{-1}, and 2% vv^{-1}, imazapyr at 280, 560 g ae ha^{-1} and 0.125% vv^{-1} plus 0.25% vv^{-1} NIS, clethodim at 280 g ai ha^{-1} and 0.25% vv^{-1} plus 2.3 L ha^{-1} COC, fluaziflop at 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS, metsulfuron at 84 g ai ha^{-1} and 29 g 60 DF formulated product 379 $liters^{-1}$ plus 0.5% vv^{-1} NIS, imazapic at 202 g ai ha^{-1} plus 0.25% vv^{-1} NIS, hexazinone at 560 and 1121 g ai ha^{-1}, MSMA at 3,700 g ai ha^{-1}, diuron at 2,200 g ai ha^{-1}, sulfosulfuron at 79 g ai ha^{-1} plus 0.5% vv^{-1} NIS, sulfometuron at 101 g ai ha^{-1}, metsulfuron + nicosulfuron at 11 + 56 g ai ha^{-1} plus 0.5% vv^{-1} NIS, and quinclorac at 841 g ai ha^{-1} plus 2.3 L ha^{-1} COC applied either summer or fall. *Miscanthus* response to these herbicide treatments were used to refine the number of treatments evaluated at the MSU Dairy in 2014. Twelve of the initial treatments were evaluated at the MSU Dairy with the addition of one new treatment, glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus

0.25% vv^{-1} NIS. According to biomass data collected at each location one year after application, glyphosate applied in the summer provided superior *Miscanthus* control compared to all other treatments, regardless of summer or fall timing. Up to 100% control was achieved at both locations when glyphosate was applied at 4,500 g ae ha^{-1} in the summer. No other herbicide nor combination of herbicides applied in the summer provided more than 50% *Miscanthus* biomass reduction a year after application. At both locations, the next most effective treatments were summer applications of sulfosulfuron at 79 g ai ha^{-1} plus 0.5% vv^{-1} NIS and glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS which provided only 41 and 43% *Miscanthus* control, respectively. *Miscanthus* control with fall applications was variable between each location. Applied in the fall, metsulfuron at 29 g 60 DF product 379 L^{-1} plus 0.5% vv^{-1} NIS provided the highest level of control at only 49% in Louisville, while *Miscanthus* control with glyphosate treatments was less than or equal to 40 percent. On ‘Freedom’ *Miscanthus* at the MSU Dairy, glyphosate at 7,300, 4,500, and 2,200 g ae ha^{-1} provided 100, 97, and 93% control with fall applications, respectively. While glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS only provided 43% control when applied in the summer, 99% control was achieved with the fall application. Results show that an application of glyphosate in the summer provides excellent control of *Miscanthus* and while results for glyphosate application in the fall varied between locations, reapplying glyphosate in the fall may be beneficial for eradication. More research is needed to test the effects of sequential applications of glyphosate in the summer and fall for *Miscanthus* control.

Nomenclature: *M. x giganteus*, Nagara; *M. x giganteus*, Freedom; Glyphosate, N-(phosphonomethyl)glycine; Imazapyr, (+/-)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid; Clethodim, (E)-(+)-2-[1-[[(3-Chloro-2-Propenyl)oxy]imino]propyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; Fluaziflop, Fluazifop-P-butyl; Metsulfuron, Metsulfuron methyl; Imazapic, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid; Hexazinone, 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione; MSMA, Monosodium methyl arsenate; diuron, 3-(3,4-Dichlorophenyl)-1; Sulfosulfuron, N-[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl]-2-(ethylsulfonyl) imidazo [1,2-a] pyridine-3-sulfonamide, Sulfometuron, Sulfometuron-methyl; Quinclorac, 3,7-Dichloroquinoline-8-carboxylic acid.

Abbreviations: NIS, nonionic surfactant; COC, crop oil concentrate.

Keywords: Glyphosate, Nagara, Freedom, biofuel crop, *Miscanthus*.

Introduction

Due to the 2007 Energy Independence and Security Act (EISA), a goal was set to decrease foreign oil consumption by producing over 79 billion L annually of advanced biofuels by 2022. To help meet this goal, the warm season perennial grass, giant miscanthus (*Miscanthus x giganteus*) from eastern Asia was introduced and promoted for production as a biofuel crop in the United States. This hybrid is reported to be sterile; however, the parents used to create this hybrid, *M. sacchariflorus* and *M. sinensis*, are both sexually fertile and highly invasive (Linde-Laursen 1993). Sterile varieties of *Miscanthus* such as ‘Freedom’ and ‘Nagara’ are also being widely promoted for biofuel production. Some potential may still exist for seed production via pollination with unrelated cultivars of *Miscanthus* near production fields of the hybrid. Producers that want to alter land use from *Miscanthus* production to other crops need consistent and effective procedures for eradication. Most research on *Miscanthus* has been focused on herbicide tolerance for establishment and production, rather than control; therefore, finding proactive control options are imperative to protect the environment against the potential threat of a new exotic, invasive weed in the landscape or facilitate land use changes.

As a biofuel crop, *Miscanthus x giganteus*is has the capacity to produce twice the biomass of switchgrass (*Panicum virgatum*) (Khanna et al. 2008) and 2.5 times the amount of ethanol per hectare compared to corn (*Zea mays*) (ScienceDaily 2008). Since 1983, extensive field trials of *Miscanthus x giganteus* have been carried out in Northern Europe. According to these trials, 2242 kg dry matter ha^{-1} year^{-1} can be produced (Schwarz et al. 1994). High yield combined with other plant characteristics, such as cold temperature tolerance, low fertility requirements, marginal land adaptability, annual harvest, low water needs, and no known insect or pathogen pests make *Miscanthus x giganteus* a potentially economical and profitable biomass crop according to some researchers (Scurlock 1999; Pyter et al. 2007). The potential to produce high yields on marginal soil with practically no production inputs not only decreases the number of hectares needed for biofuel production, but attracts the attention of farmers faced with very low to negative profit margins on other crops such as corn and soybean. However, those characteristics that make *Miscanthus x giganteus* an “excellent biomass crop candidate," also make it the excellent potential weed, according to the characteristics Baker (1974) listed for weeds.

Conflicting data regarding the production of seed by *Miscanthus x giganteus* exists in published literature. Although this plant is advertised and promoted as sterile, Nielsen (1987) found under some circumstances viable seed can be produced. Plants from these viable seed are morphologically highly variable offspring. Another researcher, Linde-Laursen (1993), concluded the production of fertile seed is rare; however, fertile seeds of *Miscanthus x giganteus* have been reported. The sterility of *Miscanthus x giganteus* nearly guarantees preservation of crops within the planted area and helps reduce the potential for movement outside that site; thus, the risk of rogue plants escaping cultivation and becoming a public nuisance is much lower than biofuels crops introduced over two centuries ago, such as Chinese tallow tree (*Triadica sebifera*) (Elliott 1824). However, due to the potential of hybridization, sterility cannot be absolutely certain. And, while it is considered an advantage to adjacent lands used for the production of other commodities and other land uses, it possesses economic disadvantages to producers. For this biofuel crop, propagation can only occur through rhizome divisions and in-vitro cultures which are expensive (Clifton-Brown and Lewandowski 2002). The high cost involved in the mass propagation of *Miscanthus x giganteus* propagules has led to the consideration of its more invasive, but seed propagated parent, *M. sinensis*. For this reason, a general control method for *Miscanthus* species must be found.

Control Methods for *Miscanthus*

Control measures for *Miscanthus x giganteus* have been proposed, including glyphosate applications (Harvey and Hutchens 1995), tillage (Powlson et al. 2005), and repeated glyphosate or fluaziflop-p applications combined with fall tillage (Speller 2003). In a greenhouse study, Anderson et al. (2011) determined that immature *Miscanthus x giganteus* shoot dry weight decreased 59% with an application of glyphosate at 3.6 kg ha^{-1}. However, in a separate field study, Anderson et al. (2011) found no significant differences between number of shoots in control plots and in each of the plots that received a single application of glyphosate at 2.5 kg ae ha^{-1} in the spring or fall. From this study, the researchers theorized that glyphosate likely did not adequately translocate to the entire rhizome mass in each plot, thus the statistical analysis failed to show significant differences of new shoot growth following glyphosate applications. They also speculated death of aboveground growth could have stimulated buds belowground to break dormancy (Anderson et al. 2011).

Generally, systemic herbicide application timing to control perennial plants is considered best when maximum basipetal transport of carbohydrates occurs (Banks et al. 1977; Mitra and Bhowmik 1999). In late fall, plants begin sending carbohydrates into the roots and rhizomes; therefore, this is considered the ideal time to maximize systemic herbicide translocation into those plant parts. *Miscanthus* produces the highest quantity of biomass by late summer or early fall. According to Beale and Long (1997), nutrients are drawn from rhizomes and translocated to aboveground plant parts from emergence until midsummer, and then are translocated back to underground plant parts as senescence occurs. During this time, carbohydrates along with the herbicides are translocated into the rhizome. However, Anderson et al. (2011) did not achieve adequate control of *Miscanthus x giganteus* with glyphosate during the fall or spring. The Roundup label[1]

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[1] Roundup Original Max label, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167

recommends glyphosate applications to perennial plants in late summer or fall or when plants initiate sexual reproduction. Along with fall and spring applications, perhaps the effectiveness of a summer application needs to be examined.

Miscanthus rhizomes occur mainly in the top 10 cm of the soil (Harvey and Hutchens 1995); therefore, mechanical control practices, such as tillage and hand hoeing, might be effective. Compared to the control plots, Anderson et al. (2011) found that shallow tillage used with two applications of glyphosate at 2.5 kg ae ha^{-1} reduced *Miscanthus* shoot numbers 67%; whereas, only a 38% reduction in shoot numbers was observed with shallow tillage plus a single glyphosate application. In this study, tillage alone resulted in a significantly higher number of shoots than plots that were tilled and sprayed with one or two applications of glyphosate. Therefore, tillage alone is not adequate for long term control. The observations from Anderson et al. (2011) support the conclusion that multiple applications of glyphosate plus tillage over a growing season, if not several growing seasons, will be necessary to eradicate a mature stand of *Miscanthus x giganteus.*

Under greenhouse conditions, tolerance studies to examine the effects of postemergence herbicides on *Miscanthus x giganteus* determined that glyphosate at 0.84 kg ae ha^{-1} caused 54% injury; therefore, a single application of glyphosate would not provide adequate control of *Miscanthus* (Everman et al. 2011). While looking at 18 postemergence herbicide treatments, Everman et al. (2011) determined that HPPD inhibitors, growth regulators, and photosystem II inhibitors caused less than 3% visual injury. Hence, herbicides in these families may be used to control unwanted vegetation in *Miscanthus* biofuel production because aboveground biomass was not significantly affected. Additionally, *Miscanthus x giganteus* and *M. sinensis* have demonstrated tolerance to preemergence and postemergence herbicide applications of the cell membrane disruptor carfentrazone and the acetolactate synthase (ALS) inhibitor halosulfuron (Smith et al. 2015). While Everman et al. (2011) reported 0% injury to *M. sinensis* from a postemergence application of nicosulfuron at 0.035 kg ai ha^{-1}, Smith et al. (2015) reported a much higher injury at 65% from the same herbicide. When evaluating rhizome propagated *Miscanthus x giganteus* under greenhouse conditions, Everman et al. (2011) and Smith et al. (2015) reported 28 and 35%, respectively, injury from a post application of nicosulfuron. Li et al. (2013) determined that nicosulfuron at 35 g ai ha^{-1} reduced *Miscanthus x giganteus* shoot height by 22% and reduced shoot dry weight 43% four weeks after treatment. To determine if nicosulfuron has the ability to control *Miscanthus* long term, sequential treatments need to be evaluated on a mature stand over a longer period of time.

Some of the most efficient management practices consist of more than one control method, such as burning followed by herbicide application, and then establish cover crops, or mowing combined with tillage. In Asia, *M. sinensis* grasslands depend on burning to maintain the floral composition of the fire dependent ecosystem, which is species diverse, and to diminish the litter layer to facilitate nutrient cycling (Iizumi 1976; Yamamoto et al. 2002). Burning stimulates annual and perennial grass growth (DiTomaso et al. 1999; Sheley et al. 1999); therefore, the use of cover crops, herbicides, or tillage, in addition to burning, may help reduce above ground biomass with invasive rhizomatous plant species.

Control Methods of Similar Perennial Plants

Similar to *Miscanthus*, giant reed (*Arundo donax L.*) is an invasive perennial grass native to eastern and southern Asia that is capable of reaching heights in excess of 6 m in warm climates. Like *Miscanthus*, giant reed was introduced as an ornamental into the United States and like cogongrass (*Imperata cylindrica*) and kudzu (*Pueraria lobata*), it was also planted for soil stabilization along drainage ditches. Giant reed can grow up to 7 cm day^{-1} and can grow more than 8 m in height after only a few months (Reiger and Kreager 1989). Giant reed is considered sterile; however, rapid growth and the capability to reproduce vegetatively has caused major weed infestations along waterways in California and across other western states (McWilliams 2004). Bell (1997) found that small pieces of giant reed break off, dislodge, and float downstream. New giant reed colonies are rapidly established where the plant rhizomes lodge in moist soil.

According to Odero et al. (2008), giant reed can be greatly suppressed by repeated mowing and tillage to deplete root and rhizome masses; however, special care must be taken to avoid the spread of rhizome fragments to un-infested areas. Mowing and tillage along the banks of waterways is not a suitable control method due to bank erosion and plant fragments spreading. Application of a systemic herbicide such as glyphosate in a 2 to 5% solution after the plant has flowered is recommended. Spencer et al. (2008) found that a late season application of glyphosate as a 3 or 5% solution was the most effective treatment to kill giant reed. Furthermore, Odero et al. (2008) recommends an application of imazapyr at 2% vv^{-1} or imazapyr at 0.5% vv^{-1} + glyphosate at 2% vv^{-1} as an effective solution. Prescribed fire can be beneficial after mechanical or chemical control methods have been utilized to remove dead biomass and stimulate the recovery of desirable species (Dudley 2003).

The success of an exotic invading species depends partly on its capability to multiply and establish rapidly in new habitats. According to Holm et al. (1977), cogongrass is the seventh worst weed in the world. Whether or not *Miscanthus* will prove to be as problematic as cogongrass is yet to be seen; however, the background history and many of the physical characteristics between these two grasses are similar. Both grasses were purposely introduced to the United States, although cultivated for different reasons. Both grasses are capable of rapid growth, survive on marginal soils, reproduce both sexually and asexually, produce wind dispersed seed, and have the ability to compete interspecifically. Several *Miscanthus* cultivars are triploids; therefore, are considered sterile. Cogongrass and cordgrass (*Spartina spp.)* are also triploids; however, the sterility broke down during rare recombination events thus allowing them to produce fertile seeds (Kyde 2010; Raghu and Davis 2007). Cogongrass has been reported to produce 3,000 seeds per culm in a single growing season (Holm et al. 1977). Thus it appears, permanent sterility cannot be certain with any plant species.

Burnell et al. (2003) demonstrated that weekly mowing of cogongrass from March through October for three consecutive years reduced the number of plants per unit area by 74%; however, mowing alone is inadequate for long term control. Like several perennial weeds, cogongrass can be difficult to control due to underground stems or rhizomes that break dormancy after top growth has been killed. Cogongrass is dependent on fire and relies on burning for dispersal and survival; therefore, burning and mowing can favor cogongrass spread from seed by removing ground litter that prevents seed contact with mineral soil (King and Grace 2000). Consequently, herbicide application combined with mechanical control methods are necessary for cogongrass eradication. Shilling et al. (1998) found that 1.12 kg imazapyr ha^{-1} provided excellent control of cogongrass one year after application. Enloe et al. (2012) found that applications of aminocyclopyrachlor, glyphosate, and imazapyr at 0.28, 4.5, and 0.84 kg ai ha^{-1} 12 months after treatment (MAT) reduced rhizome biomass by 28, 77, and 80%, respectively.

Another aggressive perennial grass that shares many characteristics of *Miscanthus* is johnsongrass (*Sorghum halepense*). According to Anderson (1999), this plant is capable of producing 80,000 seeds in a single growing season and relies on the wind, water, and animals for seed dispersal. Due to its prolific nature, johnsongrass is considered one of the world's ten worst weeds (Holm et al. 1977). Controlling johnsongrass can prove challenging because like *Miscanthus*, cogongrass, and giant reed, it too is a perennial grass capable of producing a massive rhizomatous root structure. Typically, johnsongrass rhizomes occur within the top 50.8 cm of non-compacted soil; however, rhizomes have been found as far as 1.2 m below the grounds surface (Anderson 1999). With johnsongrass, or any perennial grass, the maturity of the stand is an important factor when choosing a control method. Generally, perennial grasses are easier to control as newly emerged seedling plants that may only require a chemical treatment. Older, more established stands may need multiple chemical treatments along with a mechanical control option. For certain perennial weeds, early detection can be difficult. Johnsongrass can develop rhizomes within three to four weeks after germination (Anderson 1999).

In a three year study, Keeley and Thullen (1981) found that cultivation alone failed to prevent johnsongrass from severely reducing cotton yields. However, Gebhardt (1981) determined that PRE and POST herbicide applications in combination with cultivation increased johnsongrass control in soybean fields. Johnsongrass control can be greatly improved with yearly rotations of corn and cotton along with the rotation of different herbicide modes of action (Dale and Chandler 1979). Depending on the crop, a number of herbicides are effective for johnsongrass control. For postemergence control of johnsongrass, acetyl coenzyme A carboxylase (ACCase) and acetolactate synthase (ALS) inhibiting herbicides provided effective control in cotton and soybean (Banks and Tripp 1983; Tranel and Wright 2002). Foliar application of glyphosate and dalapon offer effective control of johnsongrass in more than 20 crops and on noncropland (McWhorter 1981). Unfortunately, excessive use of glyphosate has led to glyphosate-resistance in johnsongrass (Vial-Aiub et al. 2007) and dalapon was removed from the United States herbicide market in the early 1990s.

With limited knowledge on the biology and control of *Miscanthus*, additional research is needed before this potentially problematic plant is put into production. Countless invasive plants have agronomic or horticultural origins with extended periods of cultivation that lead to their escape, naturalization, dispersal, and negative environmental impacts on native plants and animals (Mack 2000). Two and half decades ago, it was estimated that $137 billion per year was spent on controlling plants, animals, and microbes that were introduced in the United States (Pimentel 2001). Approximately 730,000 thousand ha of United States wildlife habitat is invaded by non-native weeds each year (Pimentel 2001). In the middle 18th century, Jethro Tull (1762) wrote, “The hardest to kill are such as will grow and propagate by their seed, and also by every piece of their roots." The purpose of this research is to determine the effectiveness of herbicide treatment for control of *Miscanthus* to help minimize the potential for it to become a problematic plant like giant reed, cogongrass, or johnsongrass.

Materials and Methods

Field experiments were conducted on *Miscanthus* near Louisville, MS (33.112401, -89.010981) in 2013 and near Mississippi State University (33.394759, -88.740311) in 2014 with two objectives in mind: (1) determine the efficacy of herbicide treatments and (2) determine the effect of application timing. In 2013 these experiments were conducted on a three-year-old stand of 'Nagara' *Miscanthus*. The soil was an Ora fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Fragiudults) (NRCS 2016). The two experiments consisted of 21 herbicide or herbicide combinations applied June 25, 2013 to experiment one and September 25, 2013 to experiment two. Treatments evaluated were glyphosate at 2,200, 4,500, 7,300 g ae ha^{-1}, and 2% vv^{-1}, imazapyr at 280, 560 g ae ha^{-1} and 0.125% vv^{-1} plus 0.25% vv^{-1} NIS, clethodim at 280 g ai ha^{-1} and 0.25% vv^{-1} plus 2.3 L ha^{-1} COC, fluaziflop at 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS, metsulfuron at 84 g ai ha^{-1} and 29 g 60DF product 379 L^{-1} plus 0.5% vv^{-1} NIS, imazapic at 202 g ai ha^{-1} plus 0.25% vv^{-1} NIS, hexazinone at 560 and 1121 g ai ha^{-1}, MSMA at 3,700 g ai ha^{-1}, diuron at 2,200 g ai ha^{-1}, sulfosulfuron at 79 g ai ha^{-1} plus 0.5% vv^{-1} NIS, sulfometuron at 101 g ai ha^{-1}, metsulfuron + nicosulfuron at 11 + 56 g ai ha^{-1} plus 0.5% vv^{-1} NIS, and quinclorac at 841 g ai ha^{-1} plus 2.3 L ha^{-1} COC.

In 2014, this experiment was repeated near the Mississippi State University Dairy Unit, outside Starkville, MS on a four-year-old established stand of 'Freedom'

Miscanthus. Soil at this location was a Freeston fine sandy loam (fine-loamy, siliceous, semiactive, thermic Glossaquic Paleudalfs) and Kipling silty clay loam (fine, smectitic, thermic Vertic Paleudalfs) (NRCS 2016). The treatment list evaluated in 2014 was reduced based on lack of *Miscanthus* control observed in 2013 for some treatments. In 2014 applications of glyphosate at 2,200, 4,500, 7,300 g ae ha^{-1}, and 2% vv^{-1}, imazapyr at 280, 560 g ae ha^{-1} and 0.125% vv^{-1} plus 0.25% vv^{-1} NIS, clethodim at 280 g ai ha^{-1} and 0.25% vv^{-1} plus 2.3 L ha^{-1} COC, fluaziflop at 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS, imazapic at 202 g ai ha^{-1} plus 0.25% vv^{-1} NIS, hexazinone at 1121 g ai ha^{-1}, plus an additional combination of glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS, per Repreve Renewables[2]' personnel recommendation were applied July 11, 2014 to experiment three and September 26, 2014 to experiment four.

For both experiments both years, *Miscanthus* was mowed to a stubble height of 10 cm, then allowed to regrow to a height of 60 cm before application. Herbicides were applied in the summer and fall with a CO_2 pressurized backpack sprayer equipped with 8002VS flat fan nozzle that delivered 186 L ha^{-1} at 138 kPA. Treatments were arranged in a randomized complete block with four replications, including an untreated check, in plots 3x6 m plots. Visual control ratings on a scale of 0 indicative of no control to 100 indicative of complete control were taken 12 months after treatment (MAT). Biomass samples were harvested from a 0.3 m^2 randomly selected area of each plot 12 MAT with a reciprocating saw at the soils surface area. Biomass samples were placed in a mesh bag and dried at 58 ^{o}C for five days. Once dried, biomass samples were removed and weighed. Visual and biomass data was analyzed for variance and pooled over application

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[2] Repreve Renewables 7201 W Friendly Ave, Greensboro, NC 27419

timings. In S.A.S 9.3[3], a logarithmic transformation (base 10) of *Miscanthus* dry weight was made and the resulting data were analyzed in PROC GLIMMIX[4] (S.A.S 2011). Data for the two locations were not combined for analysis. All field data were analyzed with means separated using least square means (LSMEANS) at the 5% level of significance with the PDIFF option.

Results and Discussion

Louisville Studies

The 2013 experiments were conducted on property owned by Winston County and leased to Mendel Bioenergy[5]. Mendel planted the 'Nagara' to produce seedstock for other biofuel plantings to feed a Kior biofuel refinery near Columbus, MS. Over the duration of this experiment, Mendel Bioenergy divested their biofuel interests to Repreve Renewables, including this test site. Plant material harvested for this experiment was the first *Miscanthus* biomass harvested from the location since it was planted. However, lack of effort by both Mendel and Repreve Renewables toward biomass harvesting is a clear demonstration of one concern toward introduction of an exotic perennial grass for biofuel production. Both companies demonstrated minimal effort monitoring the site for escaped plants (Figure A3.1), but were compliant with state regulatory requirements. *Miscanthus* has already escaped cultivation in other areas (Figure A3.2). Closure of the Kior biofuel

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[3] Statistical Analysis System, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513-2414

[4] PROC GLIMMIX fits statistical models to data with correlations or nonconstant variability and where the response is not necessarily normally distributed. These generalized linear mixed models (GLMM), like linear mixed models, assume normal (Gaussian) random effects. Conditional on these random effects, data can have any distribution in the exponential family. The binary, binomial, Poisson, and negative binomial distributions, for example, are discrete members of this family. The normal, beta, gamma and chi-square distributions are representatives of the continuous distributions in this family.

[5] Mendel Bioenergy Seeds, 432 TY TY Omega Rd, Tifton, GA 31793

refinery near Columbus prior to completion is also a concern for introduction and mass planting an exotic new crop in an area without consideration of removal.

Statistical analysis of visual control ratings taken 12 MAT revealed significant differences among treatments applied in June compared to the untreated check, but no significant differences among September applications were noted (Table 3.1). Glyphosate achieved 90, 93, 85, and 85% visual control at 2,200, 4,500, 7,300 g ae ha^{-1} and as a 2% vv^{-1} solution, respectively, when applied in June; whereas, only 8, 15, 23, and 5% visual control was obtained with September applications, respectively. Hexazinone at 1121 g ai ha^{-1} achieved 45% visual control 12 MAT when applied in June. All other June applications achieved equal to or less than 28% visual control when compared to the untreated checks. All other September applications achieved equal to or less than 8% visual control when compared to untreated checks.

Statistical analysis of aboveground biomass samples taken 12 MAT revealed significant differences among treatments compared to the untreated check. The statistical analysis on shoot mass indicated, glyphosate at 2,200, 4,500, 7,300 g ae ha^{-1} or 2% vv^{-1} applied in June provided 94, 100, 85, 90% control based on shoot biomass reduction, respectively, which was significantly better than the untreated control. While some other treatments applied in June provided partial *Miscanthus* visual control, shoot biomass weights measured 12 MAT revealed no significant differences compared to the untreated control. Treatments applied in September were not consistent with those applied in June (Table 3.1). For fall applications, glyphosate applied at 4,500 g ae ha^{-1} only reduced *Miscanthus* shoot biomass 40%, compared to a 43% reduction by quinclorac at 841 g ai ha^{-1} plus 2.3 L ha^{-1} COC or 79 g ai ha^{-1} sulfosulfuron plus 0.5% vv^{-1} NIS. Metsulfuron applied at 29 g 60DF product 379 L^{-1} plus 0.5% vv^{-1} NIS provided the greatest control at 49% among September applications.

In a *Miscanthus x giganteus* field study, Anderson et al. (2011) found no significant differences between number of shoots in control plots and in each of the plots that received a single application of glyphosate at 2.5 kg ae ha^{-1} in the spring or fall. However, Anderson et al. (2011) determined that immature *Miscanthus x giganteus* shoot dry weight decreased 59% with an application of glyphosate at 3.6 kg ha^{-1} in a greenhouse study. According to biomass data in this experiment, *Miscanthus* control is highest when an application of glyphosate at 2,200 or more g ae ha^{-1} or as a 2% solution is applied in the summer as September applications failed to provide acceptable biomass control in this experiment.

Dairy Unit Studies

Statistical analysis of visual control ratings taken 12 MAT revealed a significant difference among treatments applied in July and September compared to the untreated checks (Table 3.3). Glyphosate achieved 73, 83, 95, and 45% visual control at 2,200, 4,500, 7,300 g ae ha^{-1} and as a 2% vv^{-1} solution when applied in July, respectively. Similar results were achieved in September with 73, 100, 98, and 53% visual control, respectively. Glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS provided only 15% visual control in July; whereas, 83% control was achieved from the September application. All other treatments applied in July and September achieved equal to or less than 40% visual control 12 MAT.

Statistical analysis of aboveground biomass samples taken 12 MAT revealed significant differences among treatments compared to the untreated checks. According to statistical analysis on shoot mass data, glyphosate applied at 7,300, 4,500, and 2,200 g ae ha^{-1} or 2% vv^{-1} in July achieved 100, 100, 96, and 57% control, respectively. Unlike the Louisville studies in 2013, treatments applied in July were consistent with those applied in September with the exception of the glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS which provided only 43% control when applied in the summer, but 99% control when applied in the fall based on shoot biomass measurements (Table 3.2). Fall glyphosate treatments achieved between 78 and 100% control. Although Anderson et al. (2011) did not achieve adequate control of *Miscanthus x giganteus* with glyphosate during the fall or spring, researchers theorized that glyphosate likely did not adequately translocate to the entire rhizome mass in each plot or death of aboveground growth stimulated buds belowground to break dormancy. These theories may explain why September applications at the 2013 Louisville study did so poorly.

Imazapyr at 560 g ae ha^{-1} and hexazinone at 1121 g ai ha^{-1} applied in the fall achieved 65 and 58% control, respectively. These treatments were the only non-glyphosate treatments applied in the summer or fall that provided significantly better control then the untreated checks. Biomass data showed that all other summer and fall treatments achieved less than or equal to 40% control. According to biomass data, *Miscanthus* control is highest when an application of glyphosate at 2,200 or more g ae ha^{-1} is applied in either the summer or fall.

Conclusions

For the Louisville study, application timing was significant ($P < 0.05$). Glyphosate at all four rates applied in the summer provided significantly better control then glyphosate applied in the fall compared to the untreated checks ($P<0.05$). Among all glyphosate treatments applied in the fall, 4,500 g ae/ha^{-1} provided the greatest level of control 12 MAT at 40%; whereas, between 85 and 100% control was achieved from the four different rates of glyphosate applied in the summer. For summer and fall applications, all other treatments were not significantly different according to least square means (LSMEANS) at the 5% level of significance.

At the MSU Dairy Unit study, application timing was significant ($P < 0.05$). Glyphosate at 7,300 g ae ha^{-1} applied in the summer or fall provided 100% control. For both application timings, all other non-glyphosate treatments provided less than or equal to 65% control. Compared to the summer application with 43% control, glyphosate + fluaziflop at 2,800 g ae ha^{-1} + 426 g ai ha^{-1} plus 0.25% vv^{-1} NIS had significantly better control at 99% when applied in the fall ($P<0.05$). For summer and fall applications, imazapyr at 560 g ae ha^{-1} and hexazinone at 1121 g ai ha^{-1} applied in the fall were the only non-glyphosate treatments to have significantly better control then the untreated checks ($P = 0.0109$) and ($P = 0.0453$), respectively. Glyphosate at 2% vv^{-1} applied in the summer was the only glyphosate treatment that was not significantly different from the untreated checks with 57% control ($P = 0.1327$).

Results from these experiments suggest that an application of glyphosate in the summer will provide sufficient control of *Miscanthus* 12 MAT. Although results for fall applications varied between locations in these studies, sequential glyphosate applications sprayed in the summer and fall may be beneficial in the eradication of *Miscanthus*. The differences in data collected could be a result of the two different *Miscanthus* cultivars, ‘Nagara’ and ‘Freedom’, or length of time *Miscanthus* had been established at the two locations along with other environmental factors. Further research needs to be conducted on sequential applications of glyphosate for *Miscanthus* control.

Table 3.1 *Miscanthus* visual control as affected by chemical treatments 12 months after treatment (MAT) in a field study at Louisville, MS for 2013.

Chemical	Rate	Summer	Fall
		---------Control (%)[a]--------	
Clethodim[e]	280 g ai ha^{-1}	13 FG[b]	0 H
	0.25% vv^{-1}	3 GH	0 H
Diuron	2,200 g ai ha^{-1}	0 H	0 H
Fluaziflop[c]	426 g ai ha^{-1}	28 BCD	0 H
Glyphosate	2,200 g ae ha^{-1}	90 A	8 EF
	4,500 g ae ha^{-1}	93 A	15 DEF
	7,300 g ae ha^{-1}	85 AB	23 CDE
	2% vv^{-1}	85 AB	5 FG
Hexazinone	561 g ai ha^{-1}	0 H	3 GH
	1121 g ai ha^{-1}	45 ABC	0 H
Imazapic[c]	202 g ai ha^{-1}	3 GH	0 H
Imazapyr[c]	280 g ae ha^{-1}	13 DEF	0 H
	560 g ae ha^{-1}	10 FG	8 FG
	0.125% vv^{-1}	0 H	0 H
Metsulfuron[d]	84 g ai ha^{-1}	0 H	0 H
	29 g 60DF product 379 L water^{-1}	0 H	0 H
Metsulfuron + Nicosulfuron[d]	11 + 56 g ai ha^{-1}	10 FG	0 H
MSMA	3,700 g ai ha^{-1}	0 H	0 H
Quinclorac[e]	841 g ai ha^{-1}	0 H	3 H
Sulfometuron	101 g ai ha^{-1}	8 FG	0 H
Sulfosulfuron	79 g ai ha^{-1}	0 H	0 H

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[a] Data pooled over application timing. Log$_{10}$ transformed means separated with LSMEANS and PDIFF option. Non-transformed data presented.

[b] Means followed by same letter are not significantly different from each other at 0.05 significance level.

[c] Non ionic surfactant was added at 0.25% (v/v)

[d] Non ionic surfactant was added at 0.5% (v/v)

[e] Crop oil concentrate was added at 2.3 L/ha^{-1}

Table 3.2 *Miscanthus* biomass control as affected by chemical treatments 12 months after treatment (MAT) in a field study at Louisville, MS for 2013.

Chemical	Rate	Summer	Fall
		---------Control (%)[a]-------	
Clethodim[e]	280 g ai ha^{-1}	24 DE[b]	10 A
	0.25% vv^{-1}	11 DE	25 A
Diuron	2,200 g ai ha^{-1}	23 DE	29 A
Fluaziflop[c]	426 g ai ha^{-1}	0 CDE	38 A
Glyphosate	2,200 g ae ha^{-1}	94 G	35 A
	4,500 g ae ha^{-1}	100 G	40 AB
	7,300 g ae ha^{-1}	85 F	37 A
	2% vv^{-1}	90 F	26 A
Hexazinone	561 g ai ha^{-1}	2 CDE	35 A
	1121 g ai ha^{-1}	28 E	37 A
Imazapic[c]	202 g ai ha^{-1}	11 DE	14 A
Imazapyr[c]	280 g ae ha^{-1}	0 CDE	10 A
	560 g ae ha^{-1}	0 BCD	27 A
	0.125% vv^{-1}	0 CDE	5 A
Metsulfuron[d]	84 g ai ha^{-1}	37 CDE	37 AB
	29 g 60DF product 379 L water^{-1}	0 DE	49 ABC
Metsulfuron + Nicosulfuron[d]	11 + 56 g ai ha^{-1}	22 DE	20 A
MSMA	3,700 g ai ha^{-1}	13 DE	6 A
Quinclorac[e]	841 g ai ha^{-1}	39 DE	43 AB
Sulfometuron	101 g ai ha^{-1}	0 DE	26 A
Sulfosulfuron	79 g ai ha^{-1}	41 DE	43 AB

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[a] Data pooled over application timing. Log$_{10}$ transformed means separated with LSMEANS and PDIFF option. Non-transformed data presented.

[b] Means followed by same letter are not significantly different from each other at 0.05 significance level.

[c] Non ionic surfactant was added at 0.25% (v/v)

[d] Non ionic surfactant was added at 0.5% (v/v)

[e] Crop oil concentrate was added at 2.3 L/ha^{-1}

Table 3.3 *Miscanthus* visual control as affected by chemical treatments 12 months after treatment (MAT) in a field study at the Dairy Unit, Mississippi State, MS for 2014.

Chemical	Rate	Summer	Fall
		------Control (%)[a] ---------	
Clethodim[d]	280 g ai ha^{-1}	0 C[b]	0 C
	0.25% vv^{-1}	0 C	0 C
Fluaziflop[c]	426 g ai ha^{-1}	40 B	0 C
Glyphosate + Fluaziflop[c]	2,800 g ae ha^{-1} + 426 g ai ha^{-1}	15 BC	83 A
Glyphosate[f]	2,200 g ae ha^{-1}	73 A	73 A
	4,500 g ae ha^{-1}	83 A	100 A
	7,300 g ae ha^{-1}	95 A	98 A
	2% vv^{-1}	45 A	53 A
Hexazinone	1121 g ai ha^{-1}	0 C	20 B
Imazapic[c]	202 g ai ha^{-1}	0 C	13 BC
Imazapyr[c]	280 g ae ha^{-1}	0 C	25 B
	560 g ae ha^{-1}	0 C	8 BC
	0.125% vv^{-1}	0 C	0 C

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[a] Data pooled over application timing. Log$_{10}$ transformed means separated with LSMEANS and PDIFF option. Non-transformed data presented.

[b] Means followed by same letter are not significantly different from each other at 0.05 significance level.

[c] Non ionic surfactant was added at 0.25% (v/v).

[d] Crop oil concentrate was added at 2.3 L/ha^{-1}.

Table 3.4 *Miscanthus* biomass control as affected by chemical treatments 12 months after treatment (MAT) in a field study at the Dairy Unit, Mississippi State, MS for 2014.

Chemical	Rate	Summer	Fall
		------Control (%)[a]---------	
Clethodim[d]	280 g ai ha^{-1}	38 BCD[b]	15 AB
	0.25% vv^{-1}	22 ABCD	18 AB
Fluaziflop[c]	426 g ai ha^{-1}	40 ABCD	23 AB
Glyphosate + Fluaziflop[c]	2,800 g ae ha^{-1} + 426 g ai ha^{-1}	43 ABCD	99 F
Glyphosate[d]	2,200 g ae ha^{-1}	96 FGH	93 E
	4,500 g ae ha^{-1}	100 GH	97 FG
	7,300 g ae ha^{-1}	100 H	100 H
	2% vv^{-1}	57 BCD	78 D
Hexazinone	1121 g ai ha^{-1}	0 ABCD	58 BCD
Imazapic[c]	202 g ai ha^{-1}	0 ABCD	21 AB
Imazapyr[c]	280 g ae ha^{-1}	13 ABCD	29 ABC
	560 g ae ha^{-1}	20 ABCD	65 CD
	0.125% vv^{-1}	28 ABCD	23 AB

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[a] Data pooled over application timing. Log$_{10}$ transformed means separated with LSMEANS and PDIFF option. Non-transformed data presented.

[b] Means followed by same letter are not significantly different from each other at 0.05 significance level.

[c] Non ionic surfactant was added at 0.25% (v/v).

[d] Crop oil concentrate was added at 2.3 L/ha^{-1}.

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APPENDIX A

SUPPLEMENTAL TABLES AND FIGUERS

Table A.1 Daily average ambient external greenhouse temperatures (ºC) at the Plant Science Research Center, Mississippi State University in 2014 and 2015

Date	(ºC)	Date	(ºC)
2014		2015	
2/20/2014	25	n/a	n/a
2/21/2014	13	n/a	n/a
2/22/2014	13	n/a	n/a
2/23/2014	17	n/a	n/a
2/24/2014	14	n/a	n/a
2/25/2014	13	n/a	n/a
2/26/2014	6	n/a	n/a
2/27/2014	5	n/a	n/a
2/28/2014	9	n/a	n/a
3/1/2014	18	n/a	n/a
3/2/2014	22	n/a	n/a
3/3/2014	3	n/a	n/a
3/4/2014	3	n/a	n/a
3/5/2014	11	n/a	n/a
3/6/2014	10	n/a	n/a
3/7/2014	11	n/a	n/a
3/8/2014	16	n/a	n/a
3/9/2014	18	3/9/2015	15
3/10/2014	17	3/10/2015	22
3/11/2014	20	3/11/2015	19
3/12/2014	16	3/12/2015	21
3/13/2014	12	3/13/2015	21
3/14/2014	17	3/14/2015	21
3/15/2014	19	3/15/2015	22
3/16/2014	20	3/16/2015	22
3/17/2014	9	3/17/2015	25
3/18/2014	12	3/18/2015	20
3/19/2014	17	3/19/2015	19
3/20/2014	15	3/20/2015	19
3/21/2014	17	3/21/2015	17
3/22/2014	24	3/22/2015	17
3/23/2014	15	3/23/2015	19
3/24/2014	13	3/24/2015	18
3/25/2014	11	3/25/2015	23
3/26/2014	10	3/26/2015	20
3/27/2014	19	3/27/2015	12
3/28/2014	21	3/28/2015	9
3/29/2014	17	3/29/2015	17

Table A.1 (continued)

3/30/2014	14	3/30/2015	22
3/31/2014	19	3/31/2015	23
4/1/2014	24	4/1/2015	25
4/2/2014	25	4/2/2015	26
4/3/2014	27	4/3/2015	27
4/4/2014	21	4/4/2015	17
4/5/2014	16	4/5/2015	17
4/6/2014	1	4/6/2015	21
4/7/2014	17	4/7/2015	26
4/8/2014	14	4/8/2015	27
4/9/2014	17	4/9/2015	29
4/10/2014	20	4/10/2015	23
4/11/2014	21	4/11/2015	21
4/12/2014	24	4/12/2015	22
4/13/2014	26	4/13/2015	26
4/14/2014	20	4/14/2015	25
4/15/2014	11	4/15/2015	26
4/16/2014	13	4/16/2015	25
4/17/2014	17	4/17/2015	22
4/18/2014	19	4/18/2015	22
4/19/2014	21	4/19/2015	25
4/20/2014	21	4/20/2015	21
4/21/2014	24	4/21/2015	19
4/22/2014	24	4/22/2015	24
4/23/2014	22	4/23/2015	19
4/24/2014	24	4/24/2015	20
4/25/2014	24	4/25/2015	25
4/26/2014	25	4/26/2015	26
4/27/2014	26	4/27/2015	20
4/28/2014	27	4/28/2015	17
4/29/2014	24	4/29/2015	20
4/30/2014	20	4/30/2015	22
5/1/2014	19	5/1/2015	20
5/2/2014	20	5/2/2015	21
5/3/2014	22	5/3/2015	24
5/4/2014	26	5/4/2015	25
5/5/2014	28	5/5/2015	25
5/6/2014	26	5/6/2015	26
5/7/2014	27	5/7/2015	27
5/8/2014	27	5/8/2015	28
5/9/2014	24	5/9/2015	27

Table A.1 (continued)

5/10/2014	26	5/10/2015	29
5/11/2014	28	5/11/2015	27
5/12/2014	29	5/12/2015	25
5/13/2014	28	5/13/2015	25
5/14/2014	22	5/14/2015	25
5/15/2014	18	5/15/2015	28
5/16/2014	20	5/16/2015	27
5/17/2014	22	5/17/2015	27
5/18/2014	22	5/18/2015	26
5/19/2014	26	5/19/2015	27
5/20/2014	28	5/20/2015	27
5/21/2014	29	5/21/2015	21
5/22/2014	29	5/22/2015	20
5/23/2014	29	5/23/2015	26
5/24/2014	29	5/24/2015	27
5/25/2014	29	5/25/2015	27
5/26/2014	30	5/26/2015	27
5/27/2014	28	5/27/2015	26
5/28/2014	26	5/28/2015	27
5/29/2014	26	5/29/2015	29
5/30/2014	27	5/30/2015	27
5/31/2014	27	5/31/2015	26
6/1/2014	29	6/1/2015	26
6/2/2014	28	6/2/2015	25
6/3/2014	29	6/3/2015	25
6/4/2014	30	6/4/2015	26
6/5/2014	30	6/5/2015	29
6/6/2014	30	6/6/2015	31
6/7/2014	31	6/7/2015	31
6/8/2014	29	6/8/2015	31
6/9/2014	27	6/9/2015	31
6/10/2014	25	6/10/2015	31
6/11/2014	26	6/11/2015	30
6/12/2014	29	6/12/2015	28
6/13/2014	27	6/13/2015	29
6/14/2014	28	6/14/2015	29
6/15/2014	30	6/15/2015	31
6/16/2014	30	6/16/2015	32
6/17/2014	32	6/17/2015	33
6/18/2014	31	6/18/2015	33
6/19/2014	30	6/19/2015	31

Table A.1 (continued)

6/20/2014	31	6/20/2015	32
6/21/2014	32	6/21/2015	32
6/22/2014	30	6/22/2015	33
6/23/2014	29	6/23/2015	35
6/24/2014	30	6/24/2015	30
6/25/2014	31	6/25/2015	31
6/26/2014	29	6/26/2015	32
6/27/2014	29	6/27/2015	29
6/28/2014	29	6/28/2015	28
6/29/2014	31	6/29/2015	30
6/30/2014	32	6/30/2015	29
7/1/2014	32	7/1/2015	30
7/2/2014	30	7/2/2015	31
7/3/2014	29	7/3/2015	30
7/4/2014	27	7/4/2015	27
7/5/2014	27	7/5/2015	27
7/6/2014	29	7/6/2015	29
7/7/2014	31	7/7/2015	32
7/8/2014	32	7/8/2015	32
7/9/2014	30	7/9/2015	33
7/10/2014	30	7/10/2015	32
7/11/2014	31	7/11/2015	32
7/12/2014	32	7/12/2015	34
7/13/2014	32	7/13/2015	34
7/14/2014	33	7/14/2015	34
7/15/2014	28	7/15/2015	32
7/16/2014	26	7/16/2015	33
7/17/2014	26	7/17/2015	34
7/18/2014	25	7/18/2015	34
7/19/2014	26	7/19/2015	34
7/20/2014	27	7/20/2015	35
7/21/2014	29	7/21/2015	32
7/22/2014	29	7/22/2015	31
7/23/2014	30	7/23/2015	31
7/24/2014	30	7/24/2015	31
7/25/2014	27	7/25/2015	32
7/26/2014	31	7/26/2015	32
7/27/2014	34	7/27/2015	33
7/28/2014	31	7/28/2015	34

Table A.1 (continued)

7/29/2014	26	7/29/2015	32
7/30/2014	26	7/30/2015	32
7/31/2014	27	7/31/2015	31
8/1/2014	28	8/1/2015	31
8/2/2014	29	8/2/2015	32
8/3/2014	29	8/3/2015	32
8/4/2014	30	8/4/2015	33
8/5/2014	31	8/5/2015	33
8/6/2014	31	8/6/2015	30
8/7/2014	30	8/7/2015	30
8/8/2014	31	8/8/2015	32
8/9/2014	30	8/9/2015	33
8/10/2014	31	8/10/2015	33
8/11/2014	30	8/11/2015	32
n/a	n/a	8/12/2015	31
n/a	n/a	8/13/2015	30
n/a	n/a	8/14/2015	30
n/a	n/a	8/15/2015	29
n/a	n/a	8/16/2015	29
n/a	n/a	8/17/2015	29
n/a	n/a	8/18/2015	32

Figure A.1 *Miscanthus* plant that has escaped cultivation at a Repreve Renewable's test site in 2016 at Louisville, MS

Figure A.2 *Miscanthus* escapes along Interstate 26 north near Hendersonville, NC, July 2016