EVALUATION OF HERBICIDES FOR NAPIERGRASS (*PENNISETUM PURPUREUM* SCHUM.) ESTABLISHMENT AS A CROP AND FOR CONTROL AS A WEED

By

GEORGE SHERROD CUTTS III

(Under the direction of Timothy L. Grey)

ABSTRACT

Napiergrass (*Pennisetum purpureum* Schum.) is renowned as having the highest biomass productivity among herbaceous plants, and is considered an optimum feedstock for cellulosic biofuel production. Studies indicate pendimethalin plus atrazine applied pre-emergence allows for consistently higher weed control, stem height, and dry biomass yields for establishment. Concerns about napiergrass weedy characteristics include its ability for rapid growth, robust root system, vegetative reproduction, and invasiveness to other parts of the world. Growth reduction data measured by $CO_2$ assimilation indicates that hexazinone, glyphosate, and imazapic are effective at reducing napiergrass growth. However, field results indicate glyphosate to be the most effective for control. Further research is needed on other agronomic and environmental variables in Georgia before full scale production of napiergrass can begin.

INDEX WORDS: ametryn, atrazine, biofuel, clomazone, $CO_2$ assimilation, control, diuron, establishment, flumioxazin, glyphosate, hexazinone, imazapic, imazapyr, mesotrione, metribuzin, Napiergrass, pendimethalin, sethoxydim, s-metolachlor, sulfentrazone, tembotrione, terbacil.

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B.S.A. University of Georgia, 2007

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2010

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May 2010

# DEDICATION

To Mom, Dad, and Hayley, for supporting me through the winding paths I travel. I could not have the audacity to pursue my dreams without the unending love, support, and faith that you have given me my whole life. Thank you for your trust in my endeavors and letting me spread my wings into the unknown. You are the best family that I could ever ask for.

# AKNOWLEDGMENTS

Thank you to all of my committee members, for the influence and guidance, and for opening my eyes to the wonderful field of agricultural research that allows me to pursue something much larger than myself. Dr. Lee, thank you for the generous support, and the bestowment of agricultural philosophy to challenge the way I think. Dr. Vencill, thank you for all the knowledge you have imparted upon me and instructing me through the complicated discipline of weed science. Dr. Grey and Dr. Webster, to whom I strive to emulate, thank you for the never ending advice and counsel, and inspiring me to go on to the next level.

TABLE OF CONTENTS

# LIST OF TABLES

Page

# LIST OF FIGURES

Page

# CHAPTER 1

# INTRODUCTION

With increasing demands for energy and speculative oil shortages, there is a need for alternative energy sources. Recently, scientists and policy makers have suggested cellulosic biofuel production as a practical alternative to liquid fuels derived from crude oil (EPA 2009). However, many hurdles remain to be overcome before this is a commercial reality (Rubin 2008). Napiergrass (*Pennisetum purpureum* Schum.) is noted for its high biomass yield. Also known as elephantgrass, napiergrass is a tetraploid, perennial species native to topical areas but found in subtropical areas throughout the world. Napiergrass utilizes the $C_4$ photosynthetic pathway. Studies at the USDA-ARS Coastal Plain Experiment Station in Tifton, Georgia indicate that the napiergrass variety 'Merkeron 534' is an optimum feedstock for biofuel production, in a large part for its maximized biomass yield. A clumping type perennial, napiergrass can grow to 5.5 meters tall and create up to 45 Mg $ha^{-1}$ $yr^{-1}$ of dry biomass.

There are questions about weed control during establishment of napiergrass. Autumn (August to October) planting of stems is followed by spring emergence. Napiergrass growth is vigorous once established, controlling most weeds through competition. However, a proper herbicide regime must be developed for the vulnerable time between planting and establishment. Since commercial production has been limited, there are no registered herbicides for use in napiergrass for pre- or post-planting in Georgia. Atrazine can be used pre-emergent (PRE) at planting for residual weed control during early season growth. However, there are herbicides

used for selective weed control in other *Poaceae* crops which could be used in napiergrass. Research is needed to evaluate herbicides to determine which could provide acceptable residual weed control while also having minimal napiergrass injury. Success with these herbicides on *Poaceae* crops may allow one or more to be of reasonable use in napiergrass establishment.

A concern with napiergrass has been its invasive characteristics. These characteristics include rapid growth, deep root systems, reproduction by vegetative material or by seed, efficient method of seed dispersal, ability to shade out native competitors, and resistance to grazing. It is currently listed on the invasive weed list for central and south Florida (Florida Exotic Pest Plant Council 2005), as well as in Haiti, South Africa, Hawaii, Guam, Fiji, and several other Pacific island nations (Sherley 2000). However, napiergrass does have latitudinal restrictions. In north Florida and southern Georgia for example, cooler temperatures restrict its invasiveness. Nevertheless, reservations may exist about putting napiergrass in commercial production with other crops in these regions, and control of napiergrass has been a menacing problem. It is especially difficult to control in sugarcane (*Saccharum officinarum* L.) because of the morphological similarities between the two species. For this reason, research is needed to identify napiergrass control methods in areas where it has not been previously grown to determine whether it can be utilized as a crop, the potential for rotational crop production, and its potential ecological impact.

# CHAPTER 2

# LITERATURE REVIEW

## Species History

Also known as elephantgrass, napiergrass is a tetraploid, perennial grass species found in topical and subtropical areas throughout the world. Napiergrass utilizes the $C_4$ photosynthetic pathway which is characterized by the carboxylation of phosphoneolpyruvate (PEP) and decarboxylation of $C_4$ dicarboxylic acid, which promotes increased concentrations of carbon dioxide ($CO_2$) at the bundle-sheath chloroplasts. This pathway gives a considerable advantage to $C_4$ plants over $C_3$ plants because high concentrations of $CO_2$ create a much higher stomatal resistance and thus reduce water loss through transpiration (Goodwin and Mercer 1983).

*Pennisetum* is one of the largest genera in the tribe *Paniceae*. Along with the widely cultivated cereal crop pearl millet (*Pennisetum americanum* L.), the genera consists of over 140 species world wide (Brunken 1977). Napiergrass has its origins in subtropical Africa near present day Zimbabwe, and now is introduced to most tropical and subtropical areas throughout the continent (FAO 2008). Recognized as a forage crop in the early 20$^{th}$ century, napiergrass has since been introduced to wet tropics throughout the world. Its use as a forage crop has been noted in Africa as well as Central and South America (Rainbolt 2005) and has naturalized in many of these regions (Brunken 1977).

Napiergrass was introduced to the United States in 1913 from Africa primarily as a forage crop, and in the 1960s Florida ranchers began using it as such (Woodard and Sollenberger 2008).

Having an enormous, weedy type growth (Rainbolt 2005), its forage capacity is quickly limited unless intensely grazed. Nutritive quality is also diminished with age or lack of constant grazing (Williams and Hanna 1995).

**Potential Biofuel Feedstock**

The United States is the world's largest ethanol producer (Hettinga et al. 2009). Approximately 97% of U.S. ethanol production heavily relies on starch based corn (*Zea mays* L.) for its primary feedstock (Urbanchuk 2007). In the fermentation/distillation process, corn is finely ground and water is added to form a slurry that is broken down into oligosaccharides, which are then converted to glucose. Glucose is fermented into ethanol and $CO_2$. Distillation is used to derive pure ethanol (Kwiatkowski et al. 2005). The United States produced 42 billion liters of ethanol in 2008-2009 through this process, and is mandated to produce 57 billion liters by 2015 (EPA 2009). Advanced biofuels, primarily cellulosic ethanol produced from alternative crops such as napiergrass, is expected to cover the remainder of the mandate.

Cellulosic ethanol production involves the digestion of lignocellulose of plant cell walls (Houghton et al. 2006). Pretreatment of various enzymes increases porosity of biomass particles and increases the accessibility to cellulose (Carroll and Somerville 2008). After this process, the converted glucose can be separated from the remaining solid waste, and ethanol is derived from the same fermentation process as with corn-based systems (Himmel et al. 2007). Recent advances in energy conversion strategies have improved the feasibility of producing cellulosic ethanol from high yielding crops such as napiergrass. These include advanced fermentation bacterial strains and expanding methods for generating sugars from cellulosic feed stocks (Strezov et al. 2008).

With biomass yields in excess of 45 Mg $ha^{-1}$ in a season's growth (Woodard et al. 1991), napiergrass has been widely renowned to have the highest biomass productivity among herbaceous plants (Nagasuga 2005). High efficiency levels in photosynthesis and sunlight utilization along with the ability to acclimate to environmental stresses account for the species' high productivity (Nagasuga 2005). *In vitro* testing has supported the claim that napiergrass is one of the most rapid growing plants in the world. Karlsson and Vasil (1986) reported napiergrass cell cultures were among the fastest growing cell lines when compared to other $C_4$ species. There has been renewed interest in napiergrass as an energy crop for cellulosic biofuel production. The 'Merkeron 534' variety of napiergrass (Reg. no. 119, PI-531087) grows 4 to 5 meters tall and is the preferred variety for biofuel production because of its large biomass yield (Burton 1989).

Ideal ensilage characteristics include a low buffering capacity so that less lactic and acetic acids have to be used to reach a low pH. This allows for low microbial activity that increases preservation. The low buffering capacity and adequate levels of water-soluble carbohydrates allows for efficient storage of napiergrass (Woodard et al. 1991), giving it further advantage over other biomass feed stocks.

Napiergrass also has potential for other energy sources such as various bio-gases, bio-oil, and charcoal. For example, pyrolysis studies in Brazil reported strong potential for napiergrass as an energy crop. A potential of 100 million $ha^{-1}$ of land in Brazil that is facing desertification could be used to grow napiergrass. Using a yield potential of 40 Mg of dry biomass $ha^{-1}$ $yr^{-1}$, in 2007 Brazil could have theoretically produced enough napiergrass to manufacture 4.7 times more charcoal than the world's total production, or produce enough bio-oil from napiergrass to match 45 to 55% of the world's crude oil production (Strezov et al. 2008).

**Taxonomy**

A clumping type perennial, napiergrass can create a considerable amount of biomass. Stems have a cane-like structure with leaves growing to 1.5 meters long (Rainbolt 2005). Drought tolerance allows napiergrass to be produced in deep, excessively drained sands (Woodard and Sollenberger 2008). Napiergrass has a bunch-type growth habit most common to open terrain where it is naturalized. In Georgia, napiergrass grows from early spring until first frost (March to November), with stems continually increasing in height and weight with possible accumulation of dry matter up to 5.5 meters tall. It is a stoloniferous plant, with many-noded stolons that are 1 meter and longer. Culms root at the node and reach more that 4 meters tall. Napiergrass has a stem:leaf ratio of 2:1 (Anderson et al. 2005) with thick stems that are 3-4 cm in diameter.

**Cultural Practices**

Napiergrass establishment can be achieved by seeding, but most often it is done by vegetative propagation because seeds produced by napiergrass have limited viability (Woodard and Sollenberger 2008). In the higher latitudes of the subtropics, napiergrass is best established by seed in a green house, and then vegetatively propagated in the field. Vegetative material can also be collected from established napiergrass stands. Nodal cuttings from the bottom meter of the stem have increased rooting over terminal cuttings (Hanna and Ruter 2005). Parallel rows should be furrowed 0.9 to 1.5 meters apart following a thorough preparation of the bed (Woodard and Sollenberger 2008). Stems consisting of five to ten nodes are planted in autumn and are laid in a horizontal fashion at a depth of 10 cm to improve over wintering capacity (Sladden et al. 1991). Autumn planting is followed by spring emergence. Summer planted

stems can be planted at a 45 degree angle leaving one node exposed above the soil line with the remaining nodes buried underground. This will accelerate rooting and emergence, but can inhibit the over wintering capacity for perennial use. Both methods should be completed prior to first frost. With summer and autumn plantings, harvest should not occur until the end of the following growing season to ensure winter survival (Woodard and Sollenberger 2008). Harvesting can occur 1 to 3 times per growing season, with maximum yield obtained when grown as a perennial rather than an annual-crop (Turhollow 1991). Over wintering can be inhibited when cutting close to the ground on the final harvest. Cutting napiergrass at 60 day versus 90 day intervals for multiple harvests will increase over wintering capacity (Wadi et al. 2004). The need for supplemental fertility is extremely low. For a single harvest, napiergrass should be fertilized with 70 kg to 104 kg $ha^{-1}$ in two spring split applications. When harvesting twice, make one application in the spring and one after the first harvest. For three harvests, the recommended rate should be made in three applications: one in the spring and one after each subsequent harvest (Woodard and Sollenberger 2008). Maximum yields have occurred with lowland soils that are moderately drained. However, napiergrass is extremely drought tolerant and can thrive on deep sands that are excessively drained. Woodard and Sollenberger (2008) estimate that napiergrass establishment costs \$1325 $ha^{-1}$.

There is a need for further investigation for weed control during establishment for napiergrass. Currently, there are no herbicides registered for this purpose. Atrazine can be PRE applied at planting, with glyphosate used prior to emergence in the spring for control of winter annual weeds. However, many herbicides registered for establishment of sugarcane, pearl millet, and other *Poaceae* crops do have potential for use in napiergrass because of morphological

similarities between crops. Once napiergrass is established, weed control is not an issue because it out-competes other plants (Wright 1994).

**Potential Herbicides for Establishment**

Herbicides that have the potential to be used in napiergrass establishment come from a variety of active ingredients and modes of action. Herbicides chosen for the establishment study (Table 3.1) are registered for many *Poaceae* crops such as corn, sorghum (*Sorghum* spp.), sugarcane and others. Success with these herbicides on *Poaceae* crops may allow one or more to be of reasonable use in napiergrass establishment.

Atrazine, ametryn, hexazinone, and metribuzin are triazine herbicides. This large group has a wide range of uses. Triazines are used for selective weed control in corn, sorghum, turfgrasses (*Poaceae* spp.) (LeBaron et al 2008), conifers (*Coniferae* spp.), and sugarcane (Fadayomi 1988). Selectivity is due to the presence of glutathione in tolerant species which metabolizes and detoxifies triazines (Machado et al. 1978). The mechanism of action for triazines in non-tolerant plants is considered to be inhibition of photosynthesis, and works by blocking electron transport in the photosystem II complex (Shimabukuro and Swanson 1969). Plant death occurs through the oxidation of proteins and lipids in cell membranes and organelles that causes them to desiccate (Vencill 2002).

Diuron is a substituted urea and, similar to the triazines, considered to be a photosynthesis inhibitor. Diuron inhibits the PSII electron transport chain and prevents reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) required for $CO_2$ fixation (Fayez 2000). Diuron can be PRE and post-emergent (POST) applied, and is translocated through the plant by absorption through roots to shoots via the xylem (Sedgley and Boersma 1969). Diuron is used to

selectively control weeds in corn, sorghum, and sugarcane POST applied, and is PRE applied in barley (*Hordeum vulgare*), cotton (*Gossypium hirsutum* L.), citrus (*Citrus* spp.), and ornamental trees (Vencill 2002).

Terbacil is a uracil herbicide that binds to the D1 protein and inhibits photosynthesis by blockage of the electron transport chain in photosystem II complex (Gardiner 1981; Kyle 1985). Tolerance is attributed to increased metabolism and decreased uptake (Anderson et al. 1995). Root absorbed, terbacil symptomology includes root growth inhibition and above ground plant tissue injury. Terbacil is apoplastically translocated throughout the plant in the xylem resulting in necrosis. Common uses include POST applications in alfalfa and in sugarcane (Vencill 2002).

Clomazone is an isoxazolidone, and is known to inhibit 1-deoxy-D-xyulose 5-phosphate synthase (DOXP) after being metabolized to its active form of 5-keto (Ferhatoglu and Barrett 2006). Studies have shown that this active metabolite, and not clomazone, causes phytotoxic effects in cotton (Ferhatoglu et al. 2005). Clomazone is PRE applied and root absorbed. Clomazone has a 16 day field half life in sandy-loam soils (Vencill 2002). General uses include annual broadleaf and *Poaceae* weed control in rice (*Oryza sativa*) and sugarcane (Anonymous 2005). Disulfoton is an organophosphate insecticide used in tank mixes with clomazone. Clomazone can cause severe crop injury to cotton and corn if an organophosphate insecticide is not included in a tank mix. Disulfoton inhibits clomazone from being metabolized into its active form, and results in a safening affect (Culpepper et al. 2001; Ferhatoglu et al. 2005). However, in tolerant species such as soybean (*Glycine max*), metabolism does not account for clomazone selectivity (Norman and Liebl 1989; Vencill et al. 1990; Weston and Barrett 1989). Research has not been conducted to establish if or what selective nature exists for napiergrass.

Flumioxazin is PRE and POST applied for selective broadleaf weed control in numerous crops. It is classified as an N-phenylphthalimide derivative, a group of herbicides that blocks the synthesis of chlorophyll by the inhibition of the enzyme protoporphyrinogen oxidase (PPO) (Cranmer et al. 2000; Duke et al. 1991). Selectivity of flumioxazin is through differences in metabolic rates among tolerant and susceptible plant species (Price et al. 2004). Flumioxazin is registered for sugarcane production (Anonymous 2009 B) and could have similar success in napiergrass establishment.

Sulfentrazone is a soil applied herbicide used in sugarcane production (Thomas et al. 2005). Included in the aryl triazinone chemical family, sulfentrazone is a PPO inhibitor in the chlorophyll biosynthesis pathway (Dayan et al. 1997; Duke et al. 1991). This causes an accumulation of protoporphyrin IX and production of singlet oxygen. Production of heme and chlorophyll are hence inhibited because of the blockage of this pathway (Duke et al. 1991). Sulfentrazone controls some annual grasses, small seeded broadleaf weeds, *Amaranthus* spp., as well as many broadleaf weeds in peanut (*Arachis hypogaea*) production in the southeastern United States (Collins et al. 2001; Hulting et al. 2001; Niekamp and Johnson 2001; Webster 2001). However, it is not currently registered for use in peanut production in Georgia. Sulfentrazone is also used for weed control in sugarcane, sorghum, corn, soybean, cotton, tobacco (*Nicotiana spp.* L.), and turfgrasses (Anonymous 2009 A).

The triketone chemical family includes the herbicides mesotrione and tembotrione. Triketone herbicides are considered to be bleaching herbicides in that the symptoms in susceptible plant species include foliar bleaching followed by necrosis. This is due to the inhibition of p-hydroxyphenyl pyruvate dioxygenase (HPPD) that is involved in the synthesis of carotenoids (Mitchell et al. 2001; Norris et al. 1998). Selectivity is through more rapid

metabolism or lower rates of foliar uptake in tolerant plants (Bartlett and Hall 2000; Mitchell et al. 2001).

*S*-metolachlor is in the chloroacetanilide herbicide family that inhibits the bio synthesis of several key plant components including proteins, lipids, flavonoids, and fatty acids (LeBaron et al. 1988). *S*-metolachlor specifically inhibits very long chain fatty acid (VLCFA) synthesis (Böger 2003). Metolachlor and *s*-metolachlor are equivalent, but a breakthrough in the manufacturing of the chemical allowed for a selective production of the more active *s*-isomer. Although all plants metabolize *s*-metolachlor, selectivity seems to be based on the timing and or speed of the metabolism (Kearney 1976). Uses in corn and sorghums make *s*-metolachlor practical for possible use in napiergrass applied PRE for residual weed control during establishment.

Pendimethalin is a dinitroaniline herbicide, and inhibits key processes during cell mitosis by binding to the microtubule protein tublin (Vaughn and Lehnen 1991). Pendimethalin is PRE applied and primarily root absorbed (Appleby and Valverde 1989), but can be also be coleoptile absorbed which seems to be the more sensitive absorption site in grasses (Barrentine and Warren 1971). Tolerance in species is thought to be due to differences in metabolism and sequestration of pendimethalin within the lysigenous glands (Shaner et al. 1998). Pendimethalin has been shown to have up to a 69 day residual with 85% weed control (Mueller-Warrant 1999), and is registered for control of annual grasses and certain broadleaf weeds in various *Poaceae* crops (Anonymous 2007).

**Weedy Characteristics**

In the lower latitudes of the tropical and sub-tropical United States, napiergrass is considered a noxious weedy species. Napiergrass is also an invasive weed in tropical regions because of its rapid growth rate and robust rhizomous vegetative reproduction. Napiergrass is considered invasive in Hawaii, Guam, Fiji, and several other Pacific island nations, as well as Haiti and South Africa (Sherley 2000). It has been listed as an invasive species by the Florida Exotic Pest Plant Council (Florida Exotic Pest Plant Council 2005). However, napiergrass is described as having latitudinal restrictions to its invasive capacity above 30 degrees latitude north and south. For example, in north Florida and southern Georgia, cooler temperatures restrict its invasive potential by inducing winter dormancy. Frost kills above ground herbage, though soil must be frozen to kill below ground rhizomes (Duke 1983). The species also only flowers under short days and will not produce seed where winter temperatures reach 0°C or below, thus losing its invasive potential in latitudes north of the Florida pan-handle (Hanna and Ruter 2005).

Napiergrass was introduced to the United States as a forage crop. Although its use in this capacity has diminished, napiergrass has since become a major weed problem where it was introduced. The problem was further exacerbated when areas once established with napiergrass for forage were cultivated for other crops and vegetative material was unintentionally dispersed to surrounding areas. The species began to spread into adjacent fields and road banks, especially in lower latitudes such as south Florida where winter dormancy does not restrict growth (Rainbolt 2005). However, there is limited information regarding to what extent napiergrass can survive cooler temperatures, or what effect climates similar to north Florida and southern Georgia may have on the potential for napiergrass to become an invasive species.

**Current Control Methods**

Napiergrass control has been a menacing problem in many parts of the world (Sherley 2000). It is especially difficult to selectively control in sugarcane due to morphological similarities between the two species. Cultural control of napiergrass is an inexpensive method of control. However mechanical control can increase infestations by disturbing and dispersing rhizomes into non-infested areas. Plants should be totally removed because of their vegetative reproductive characteristics (Rainbolt 2005).

Control of napiergrass before it can overwhelm a sugarcane crop is important. There are no herbicides currently labeled for selective napiergrass control in sugarcane, therefore spot treatments of non-selective herbicides is the common management strategy (Hutchinson et al. 2007). Current napiergrass control methods include repeated, timely applications of glyphosate. Glyphosate is non-selective, foliar applied, and is extremely effective on perennial grass species (Brown et al. 1988; Parochetti et al. 1975; Prostko et al. 2006; Richard 1991). Its mode of action is the inhibition of amino acid production synthesis on the shikimic pathway (Amrhein et al. 1980). Glyphosate is recommended at a 1080 g a.e $L^{-1}$ solution rate for control of napiergrass (Rainbolt 2005). However, *in vitro* tests on the embryogenic callus of napiergrass with low rates of glyphosate ($\leq$ 0.005 g a.e. $L^{-1}$ solution) were not inhibitive of callus formation (Rajasekaran et al. 1986).

Other grass control herbicides could be effective for napiergrass control. Imazapic and imazapyr, both imidazolinone (IMI) herbicides, inhibit enzyme production by binding to acetolactate synthase (ALS) on the branched-chain amino acid biosynthetic pathway (Saari et al. 1994; Shaner et al. 1984; Zhao et al. 1999). Imazapic is registered for control of annual and perennial grass species in peanuts (Burke et al. 2004; Grey et al. 2003; Jordan and York 2002)

and for weed control during establishment of legumes and wildflowers in grasslands (Beran et al. 1999A; 1999B). Imazapyr is used in tank mixes with glyphosate or alone to control cogongrass (*Imperata cylindrica* L.), an invasive perennial grass species in the southeastern United States (Faircloth 2005).

Hexazinone inhibits photosynthesis by blocking electron transport in the photosystem II complex (Shimabukuro and Swanson 1969). Data indicates that hexazinone reduces cumulative bermudagrass (*Cynodon dactylon* L.) yields (Wilder et al. 2008) and reduces overall bahiagrass (*Paspalum notatum*) biomass accumulation (Sellers et al. 2008). These are also $C_4$ perennial species, and thus hexazinone could have similar inhibitory effects on napiergrass.

**Objectives**

There has been interest in establishing napiergrass as an energy crop for bio-fuel production. However, there is a lack of information concerning weed control during establishment of napiergrass for the period after emergence but prior to plants becoming mature enough to out-compete weeds. The first objective of this research is to determine the most practical herbicide (or combination of herbicides) that will provide effective residual weed control in early to mid-spring to allow napiergrass plants to become established. A broad spectrum of herbicides will be researched and efficacy on napiergrass growth and overall biomass yield evaluated.

Napiergrass has been noted as being the fastest growing plant among herbaceous species and as having extremely efficient photosynthetic rates, giving the plant strong weedy potential. Thus, the second objective is to evaluate photosynthesis to determine herbicide effect on growth reduction by measuring $CO_2$ assimilation in napiergrass using an open-flow gas exchange

system. Research will be conducted to determine if herbicides will be effective in reducing or halting napiergrass growth.

The potential invasiveness of napiergrass either as a plant that escapes cultivation or in subsequent rotational crops in temperate regions is a major concern that requires research prior to full scale production. The ability to effectively control napiergrass is crucial to understand how and where the species can be planted and utilized as a biofuel crop. Fully established, napiergrass is difficult to control due to its stoloniferous growth habit. Thus the third objective is to evaluate herbicides that are effective in controlling napiergrass as an unwanted species in Georgia.

# CHAPTER 3

# HERBICIDE EFFECT ON NAPIERGRASS (*PENNISETUM PURPUREUM SHUM.)* ESTABLISHMENT[1]

[1] Cutts, G.S., T.L. Grey, R.D. Lee, T.M. Webster, R.S. Tubbs, W.F. Anderson, and W.K. Vencill. Submitted to Crop Management.

**Abstract:** Napiergrass is a rapidly growing species under consideration for cellulosic biofuel production. However, information about agronomic production of napiergrass as a crop is lacking. Weed control and crop response information will be required for successful production. Field screening studies were initiated to evaluate pre-emergence herbicides for weed control and napiergrass tolerance. Studies were initiated at Plains and Fort Valley Georgia. Napiergrass was established from vegetative stock planted in the autumn 2008. The adapted planting stock from an established napiergrass stand was obtained from the Coastal Plains Experiment Station at Tifton, Georgia. Herbicides included atrazine, clomazone, diuron, pendimethalin, metribuzin, sulfentrazone, flumioxazin, ametryn, *s*-metolachlor, mesotrione, tembotrione, hexazinone, and terbacil. These were selected based on their common use for selective weed control in numerous *Poaceae* crops while possessing several modes of action. Treatments were applied prior to napiergrass emergence in March 2009 at both locations. Napiergrass emerged 2 to 3 weeks after application (WAA). Data collection included napiergrass stand counts, plant height, and biomass yield. At 12 WAA, average stand counts were 1.0 plant $m^{-2}$ at Fort Valley and 0.4 plant $m^{-2}$ at Plains across all treatments. Pendimethalin plus atrazine treated napiergrass had a height of 32 cm, greater than the non-treated control (23 cm). Atrazine, clomazone, diuron, pendimethalin, flumioxazin, ametryn, *s*-metolachlor, mesotrione, tembotrione, and hexazinone had greater stem heights than the non-treated control (23 cm) at 12 WAA. Metribuzin and sulfentrazone treated napiergrass had stem heights equal to the non-treated control. By 15 WAA, napiergrass had recovered from any herbicide injury and there were no height differences. Napiergrass treated with pendimethalin plus atrazine yielded 55 and 86 Mg $ha^{-1}$ at Plains and Fort Valley, respectively. The non-treated control yield was 53 Mg $ha^{-1}$ at both locations.

**Introduction**

With increasing demands for energy, a need for alternate sources of energy is rising. Recent emphasis is on evaluating cellulosic biofuel production as a practical alternative to crude oil-based liquid transportation fuels. Napiergrass is noted for its especially high biomass yields and has gained interest as a possible feedstock for cellulosic biofuel production (Duke 1983). Also known as elephantgrass, napiergrass is a tetraploid, $C_4$ perennial grass species found in topical and subtropical areas throughout the world. With biomass yields in excess of 45 Mg $ha^{-1}$ in a full season's growth (Woodard et al. 1991), napiergrass has been widely renowned to have the highest biomass productivity among herbaceous plants (Nagasuga 2005). This high level of productivity is attributed to its efficient conversion of $CO_2$ to cellulose during photosynthesis. Napiergrass has unique spacial and physical properties of the bundle sheath cell wall, as compared to other $C_4$ species, that allows for minimal $CO_2$ diffusion, or leakage, resulting in greater $CO_2$ saturation at ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) (von Caemmerer and Furbank 2003).

Studies indicate that the napiergrass variety 'Merkeron 534' is an optimum feedstock for biofuel production due to its maximized biomass yield (Burton 1989). There are questions about weed control during establishment of napiergrass in Georgia. Autumn planting allows the plant to emerge the following spring and have a full season's growth as opposed to planting in mid-summer. Because napiergrass seed have limited viability, it is most often vegetatively propagated. Typically five to ten nodes of a stem are planted horizontally in a row at a depth of about 10 centimeters. Plant growth is vigorous enough once established to control most weeds, but a proper herbicide regime must be developed for the vulnerable time between planting and establishment.

Since commercial production has been limited, there are no PRE or POST herbicides registered for napiergrass production in Georgia. Research has indicated that atrazine can be used PRE before napiergrass emergence in the spring for control of winter annual weeds (Bogdan 1977). There are many herbicides available for selective weed control in *Poaceae* crops that are morphologically similar to napiergrass. Therefore, herbicides need to be evaluated to determine which could provide acceptable napiergrass establishment. The herbicides chosen for the screening tests, listed in Table 3.1, are labeled for corn, sorghum, sugarcane, and others. Success with these herbicides on *Poaceae* crops may allow for identification and potential registration for use in napiergrass establishment if this crop is to be produced for biofuel.

**Materials and Methods**

'Merkeron 534' napiergrass was planted for both locations at Plains and Fort Valley, Georgia in October of 2008. Planting stock was taken from an established napiergrass stand at the Coastal Plains Experiment Station in Tifton, Georgia. Stems were cut from the bottom of the plant that contained 5 to 10 viable nodes. Plots were in bedded, conventionally tilled fields. Soils were a Faceville sandy loam (fine, kaolinitic, thermic Typic Kandiudults) at Plains and a Dothan sandy loam (fine loamy, siliceous, thermic, Plinthic Paleudult) at Fort Valley. A trenching implement was used to plow a single planting row to a 10 cm depth through the center of the bedded soil. Stems were then laid horizontally, end to end, in the planting trench and then covered by hand and rotor-packed for adequate soil contact with vegetative material, followed by irrigation. Plots were made 1.8 meters wide by 6 meters long. Plants emerged two to three weeks after planting. Fertilizer (10 – 10 – 10) was applied at 300 kg/ha in March of 2009 after first emergence at Plains. No supplemental fertilizer was applied at Fort Valley.

Plots were fallow for the winter of 2008/2009. Prior to treatments and napiergrass emergence, a broadcast application of glyphosate[1] (0.84 kg a.e. $ha^{-1}$) was made at each location to control winter annual weeds. On March 11 2008, treatments were applied at both locations using a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$. Treatments included atrazine[2], atrazine plus crop oil concentrate[3] (COC), clomazone[4], clomazone plus disulfoton[5], diuron[6], diuron plus non-ionic surfactant[7] (NIS), pendimethalin[8], pendimethalin plus atrazine, metribuzin[9], sulfentrazone[10], flumioxazin[11], ametryn[12], *s*-metolachlor[13], mesotrione[14], tembotrione[15], hexazinone[16], terbacil[17] and a non-treated control (Table 3.1). Herbicide rates corresponded to the recommendations for labeled uses in respective *Poaceae* crops (Table 3.2).

In June and July of 2009, summer annual weed pressure increased as herbicide efficacy diminished. In Fort Valley, plots were located in an old pasture, and the plant population that encroached on the plots was representative of typical pasture annuals including clovers (*Trifolium* spp.), plantains (*Musaceae* spp.), and grasses (*Poaceae* spp.). Alleys and row middles were mowed at Fort Valley on May 28, 2009. On June 10, 2009, post-directed applications of 2,4-D[18] and atrazine at 1.2 L product $ha^{-1}$ and 2.4 L product $ha^{-1}$, respectively, were applied with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$ to row middles for suppression of broadleaf and grass weeds.

Visual weed control ratings (0% to 100% with 0% = no control and 100% = total control) were collected in Plains 6 weeks after application (WAA). These measures were not practical in Fort Valley because of the plant biodiversity present. Weeds present at Plains included Palmer amaranth (*Amaranthus palmeri*), Texas millet (*Urochloa texanum*), and yellow nutsedge (*Cyperus esculentus*). To suppress this weed population present in mid season, post-directed applications were made to the row middles on June 10, 2009 of atrazine, 2,4-D, and bentazon[19]

plus COC (0.25% v/v) , both at 2.4 L product $ha^{-1}$. On July 27, 2009 a post-directed application of sethoxydim[20] plus COC was made at 2.4 L product $ha^{-1}$. All applications were made with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$.

Stand counts were made at each location 4, 6, and 12 WAA, which included counts of emerged stems in each plot. Height measures were taken 12, 14, 16, and 20 WAA, which included two stem heights within each plot. In January of 2010, plots were harvested for biomass. This timing of harvest allowed for maximum dry-down of the plants and would represent harvest timing for cellulosic biofuel feedstock production (Sumner and Hellwig 1988). One meter row of each plot was harvested using a chain saw. Plant material was then tied into bundles and weighed. To acquire moisture samples from each site, whole plant material was randomly selected, then chopped and consolidated. Sub-samples were taken for fresh weights, and then dried for 48 hours at 30°C. Dry weight of the sub-sample was then determined. From this, percent moisture content was calculated for each site to derive dry-weight biomass yield.

Growing degree days (GDD) were calculated for both locations by the equation

$$GDD = \left( \frac{\max °C + \min °C}{2} \right) - t_b \qquad [1]$$

where $t_b$ is base temperature for napiergrass growth (12°C) (Allison et al. 2006). GDD included the period from the last spring frost to the first autumn frost in 2009 which was March 6 to December 5 and April 8 to November 26 for Plains and Fort Valley, respectively.

**Experimental Design**

There were two site locations at Plains and Fort Valley, Georgia. Experiments were arranged as a randomized complete block design with four replications of treatments. Data were subjected to analysis of variance (SAS Inst. 2001). Treatment means were separated using Fisher's

Protected LSD ($P \leq 0.05$). Means separation were performed on transformed data, but original treatment means were presented for clarity.

**Results and Discussion**

There were no significant differences in napiergrass stand population among treatments, indicating that none of the herbicides affected napiergrass establishment at either location (Table 3.2). At 12 WAA, stand populations for all treatments at Fort Valley were 1.0 $m^{-2}$; at Plains the stands for all treatments were 0.4 $m^{-2}$. Napiergrass dormancy began after the first freeze which occurred on October 28, 2008 at Plains and November 17, 2008 at Fort Valley (Data not shown). This earlier freeze at Plains could have contributed to lower stand establishment. Frost kills above ground herbage and frozen soil will kill below ground napiergrass rhizomes (Duke 1983). If planting material did not have adequate soil contact, a lack of protection from cold temperatures could have resulted in plant kill at Plains. However, data from screenings done by Turhollow (1991) indicate that napiergrass plants in Alabama and Tennessee all survived the first winter after planting.

At Plains, high levels of Palmer amaranth and Texas millet control (98% and 93% respectively) were observed 6 WAA with pendimethalin plus atrazine (Table 3.3). Jones and Griffin (2009) also reported high levels (>90%) of early season weed control with pendimethalin plus atrazine in sugarcane. Residual control of yellow nutsedge was reduced 6 WAA (Table 3.3), with sulfentrazone, terbacil, and mesotrione providing the highest levels of control (85%, 84%, and 78% respectively). Previous data has indicated that sulfentrazone and terbacil provide residual yellow nutsedge control when applied pre-emergent, while control with mesotrione is variable (Wehtje et al. 1997; Yang 1978; Johnson et al. 2002).

No significant treatment by location interaction existed for stem height data, therefore data were combined over location for discussion (Table 3.4). At 12 WAA, there were differences in stem height among treatments. Pendimethalin plus atrazine treated napiergrass had a height of 32 cm, greater than the non-treated control (23 cm) (Data not shown). This was attributed to effective weed control, reducing competition with napiergrass. This is similar to observations reported by Jones and Griffin (2009). Terbacil reduced napiergrass height (22 cm) relative to the non-treated control at 12 WAA which corroborates previous studies with terbacil which injured sugarcane (Dissannayake et al. 1998). Napiergrass treated with atrazine, atrazine plus COC, clomazone, clomazone plus disulfoton, diuron, diuron plus NIS, pendimethalin, flumioxazin, ametryn, *s*-metolachlor, mesotrione, tembotrione, and hexazinone had greater stem height than the non-treated control at 12 WAA. Higher levels of weed control with these herbicides could attribute to more robust napiergrass growth. Metribuzin and sulfentrazone reduced stem height (22.8 cm) as compared to the non-treated control (Table 3.4). By 15 WAA, all plants recovered from any stem height reduction sustained from weed competition or herbicide injury (Data not shown).

Due to a lack of a significant treatment by location interaction, biomass yield data were combined for analysis. Although there were no significant differences among treatments for biomass yield, there were significant differences in biomass yield between locations ($F = 6.08$, $P > .0152$) (Table 3.5). Among all treatments mean dry biomass yield was 35.8 Mg $ha^{-1}$ at Plains, while at Fort Valley mean dry biomass yield averaged 24.5 Mg $ha^{-1}$ among all treatments. These differences can be attributed to agronomic and environmental factors not analyzed in this study. Fort Valley was a low input site with no irrigation or fertilizer applications, whereas Plains had regular irrigation and was well fertilized. However, a correlation may exist between a differing

number of growing degree days (GDD) calculated for napiergrass at each site (Table 3.6). Data from Allison et al. (2006) indicates that napiergrass leaf production increases linearly with temperature.

**Conclusions**

Napiergrass treated with pendimethalin plus atrazine had consistently greater dry biomass yields. Greater weed control was also observed, resulting in napiergrass with greater stem heights. A continuation of this study is needed before herbicides for establishment in napiergrass can be recommended. Further analysis on other agronomic and economic establishment factors could determine the best herbicide option. POST weed control was also necessary during this study. Observations made with subsequent POST applications made in this study show strong potential for excellent weed control and crop tolerance to napiergrass. Further research is needed to explore these and other post emergent weed control options.

Table 3.1: Herbicides applied for napiergrass establishment at Plains and Fort Valley, GA in 2009[a]

| Active Ingredient (ai) | Chemical Family | Mode of Action |
|---|---|---|
| atrazine | triazine | photosynthesis inhibitor |
| ametryn | triazine | photosynthesis inhibitor |
| clomazone | isoxazolidone | DOXP inhibitor |
| diuron | substituted urea | photosynthesis inhibitor |
| flumioxazin | N-phenylphthalimide | PPO inhibitor |
| hexazinone | triazine | photosynthesis inhibitor |
| mesotrione | triketon | HPPD inhibitor |
| *s*-metolachlor | chloroacetanilide | VLCFA inhibitor |
| metribuzin | triazine | photosynthesis inhibitor |
| pendimethalin | dinitroaniline | mitotic cell division inhibitor |
| sulfentrazone | aryl triazinone | PPO inhibitor |
| tembotrione | triketon | HPPD inhibitor |
| terbacil | uracil | photosynthesis inhibitor |

[a]Field experiments located in Plains and Ft. Valley, Georgia.

Table 3.2: Stand population 12 weeks after application for herbicides applied for napiergrass establishment at Plains and Fort Valley, GA in 2009[a]

| Treatment. | Rate | Stand Counts | |
|---|---|---|---|
| | | Plains | Fort Valley |
| | kg ai ha$^{-1}$ | sq. m$^{-1}$ | |
| atrazine | 3.3 | 0.5 | 0.9 |
| atrazine plus COC | 3.3 + 1% (v/v) | 0.3 | 1.2 |
| clomazone | 1.4 | 0.3 | 0.7 |
| clomazone plus disulfoton | 1.4 + 1.1 | 0.5 | 0.8 |
| diuron | 2.8 | 0.4 | 0.8 |
| diuron plus NIS | 2.8 + 0.25% (v/v) | 0.3 | 1.3 |
| pendimethalin | 2.8 | 0.4 | 1.1 |
| pendimethalin plus atrazine | 2.8 + 3.3 | 0.5 | 1.1 |
| metribuzin | 2.2 | 0.3 | 1.2 |
| sulfentrazone | 0.4 | 0.2 | 1.1 |
| flumioxazin | 0.3 | 0.2 | 1.0 |
| ametryn | 2.2 | 0.4 | 0.8 |
| *s*-metolachlor | 1.6 | 0.2 | 0.7 |
| mesotrione | 1.4 | 0.5 | 1.0 |
| tembotrione | 1.4 | 0.5 | 1.3 |
| hexazinone | 0.2 | 0.5 | 1.1 |
| terbacil | 0.9 | 0.3 | 1.3 |

[a]No significant treatment by location interaction existed; therefore data were combined over location for presentation.
[b]ANOVA indicated that there were no differences among treatment means.

Table 3.3: Weed control 6 weeks after application for herbicides applied for napiergrass establishment at Plains, GA in 2009[a]

| Treatment. | Rate | P. amaranth | | T. millet | | Y. nutsedge | |
|---|---|---|---|---|---|---|---|
| | kg ai $ha^{-1}$ | % | | | | | |
| atrazine | 3.3 | 78 | abc | 53 | cde | 24 | ef |
| atrazine plus COC | 3.3 + 1% (v/v) | 73 | abc | 30 | e | 15 | f |
| clomazone | 1.4 | 60 | bc | 36 | e | 23 | ef |
| clomazone plus disulfoton | 1.4 + 1.1 | 45 | c | 45 | de | 11 | f |
| diuron | 2.8 | 75 | abc | 80 | abc | 50 | bcd |
| diuron plus NIS | 2.8 + 0.25% (v/v) | 93 | ab | 80 | abc | 35 | def |
| pendimethalin | 2.8 | 93 | ab | 98 | a | 30 | def |
| pendimethalin plus atrazine | 2.8 + 3.3 | 98 | a | 93 | ab | 46 | cde |
| metribuzin | 2.2 | 99 | a | 90 | ab | 70 | abc |
| sulfentrazone | 0.4 | 98 | a | 75 | abcd | 85 | a |
| flumioxazin | 0.3 | 99 | a | 61 | bcde | 30 | def |
| ametryn | 2.2 | 90 | ab | 51 | cde | 36 | def |
| *s*-metolachlor | 1.6 | 88 | ab | 58 | cde | 74 | ab |
| mesotrione | 1.4 | 81 | ab | 70 | abcd | 78 | a |
| tembotrione | 1.4 | 100 | a | 83 | abc | 69 | abc |
| hexazinone | 0.2 | 63 | bc | 73 | abcd | 28 | def |
| terbacil | 0.9 | 100 | a | 90 | ab | 84 | a |

[a]Values followed by the same letter in each column do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

Table 3.4: Stem height at12 weeks after application for herbicides applied for napiergrass establishment at Plains and Fort Valley, GA in 2009[a]

| Treatment. | Rate | Stem Height | |
|---|---|---|---|
| | kg ai $ha^{-1}$ | cm | |
| atrazine | 3.3 | 30 | bc |
| atrazine plus COC | 3.3 + 1% (v/v) | 28 | c |
| clomazone | 1.4 | 27 | c |
| clomazone plus disulfoton | 1.4 + 1.1 | 27 | c |
| diuron | 2.8 | 32 | ab |
| diuron plus NIS | 2.8 + 0.25% (v/v) | 28 | bc |
| pendimethalin | 2.8 | 27 | c |
| pendimethalin plus atrazine | 2.8 + 3.3 | 32 | a |
| metribuzin | 2.2 | 23 | de |
| sulfentrazone | 0.4 | 23 | de |
| flumioxazin | 0.3 | 25 | d |
| ametryn | 2.2 | 27 | c |
| *s*-metolachlor | 1.6 | 27 | c |
| mesotrione | 1.4 | 30 | bc |
| tembotrione | 1.4 | 27 | c |
| hexazinone | 0.2 | 30 | b |
| terbacil | 0.9 | 22 | e |

[a]No significant treatment by location interaction existed; therefore data were combined over location for presentation.
[b]Values followed by the same letter do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

Table 3.5: Napiergrass dry biomass yield for herbicides applied for napiergrass establishment at Plains and Fort Valley, GA in 2009[a]

| Treatment. | Rate | Dry biomass yield | |
|---|---|---|---|
| | | Plains | Fort Valley |
| | kg ai $ha^{-1}$ | Mg $ha^{-1}$ | |
| atrazine | 3.3 | 55 | 33 |
| atrazine plus COC | 3.3 + 1% (v/v) | 59 | 33 |
| clomazone | 1.4 | 54 | 37 |
| clomazone plus disulfoton | 1.4 + 1.1 | 33 | 47 |
| diuron | 2.8 | 82 | 39 |
| diuron plus NIS | 2.8 + 0.25% (v/v) | 33 | 37 |
| pendimethalin | 2.8 | 46 | 55 |
| pendimethalin plus atrazine | 2.8 + 3.3 | 55 | 86 |
| metribuzin | 2.2 | 47 | 27 |
| sulfentrazone | 0.4 | 67 | 46 |
| flumioxazin | 0.3 | 56 | 29 |
| ametryn | 2.2 | 57 | 30 |
| s-metolachlor | 1.6 | 36 | 30 |
| mesotrione | 1.4 | 43 | 56 |
| tembotrione | 1.4 | 65 | 51 |
| hexazinone | 0.2 | 58 | 37 |
| terbacil | 0.9 | 39 | 59 |

[a]Biomass yields were collected on January 12, 2010 at Fort Valley, and January 13, 2010 at Plains.

[b]ANOVA indicated that there were no differences among treatment means.

Table 3.6: Napiergrass dry biomass yield and Growing Degree Days for Plains and Fort Valley, GA in 2009[a]

| Site Location | Mean dry-biomass yield | | GDD[d] |
|---|---|---|---|
| | Mg ha$^{-1}$ | | |
| Plains | 36 | a | 4280 |
| Fort Valley | 25 | b | 4162 |

[a]Data analysis indicated there were no differences among treatment, therefore data were presented by location.

[b]Values followed by the same letter do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

[c]Moisture samples indicated 62.5% moisture at Plains, and 57.1% moisture at Fort Valley.

[d]Growing Degree Days calculated from last spring frost to first autumn frost in 2009 (March 6 to December 5 at Plains; April 8 to November 26 at Fort Valley) by the equation $GDD = \left(\frac{\max °C + \min °C}{2}\right) - t_b$ ,where $t_b$ is base temperature for napiergrass growth (12°C).

# CHAPTER 4

# HERBICIDE EFFECT ON NAPIERGRASS (*PENNISETUM PURPUREUM* SCHUM.) GROWTH MEASURED BY $CO_2$ ASSIMILATION AND STOMATAL CONDUCTANCE[2]

[2] Cutts, G.S., T.L. Grey, R.D. Lee, T.M. Webster and W.K. Vencill. To be submitted to Weed Science.

**Abstract:** Experiments were conducted to determine the effect of herbicide treatments on napiergrass growth by measuring carbon dioxide ($CO_2$) assimilation and stomatal conductance. Napiergrass stems containing three lateral nodes were planted in pots and greenhouse grown. Two weeks prior to herbicide treatment, plants were pruned to uniform size. Hexazinone, glyphosate, and imazapic were applied at 200, 869, and 70 g ai $ha^{-1}$, respectively, with a non-treated control. Carbon dioxide assimilation and stomatal conductance were measured using an open flow gas exchange system at 1 day prior to treatment, and 1, 2, 3, 5, 7, 9, and 12 days after treatments (DAT) for hexazinone and glyphosate, and up to 22 DAT for imazapic. Declines in $CO_2$ assimilation and stomatal conductance of 93 and 82% occurred for hexazinone at 1 DAT, respectively. Glyphosate and imazapic $CO_2$ assimilation and stomatal conductance were reduced 97 and 75% at 10 DAT, respectively. All herbicide treatments were different from one another and the non-treated control. By 12 DAT, all hexazinone and glyphosate treatments had no $CO_2$ assimilation or stomatal conductance, indicating plant death. Imazapic treatments reached constant low rates by 22 DAT. Further studies are needed to confirm these results in the field.

## Introduction

Napiergrass, also known as elephantgrass, is a tetraploid $C_4$ perennial grass species found in tropical and subtropical areas throughout the world (Brunken 1977). In lower latitudes of the tropical and sub-tropical United States, napiergrass is considered a weedy species (Mossler 2008). Napiergrass is considered an invasive weed species in tropical regions because of its rapid growth rate and robust rhizomous vegetative reproduction. It has been listed as an invasive species by the Florida Exotic Pest Plant Council (Florida Exotic Pest Plant Council 2005). However, Hanna and Ruter (2005) describe napiergrass as having latitudinal restrictions above 30 degrees latitude north and south, such as in north Florida and southern Georgia where cooler temperatures restrict its invasive potential. The species only flowers under short days and will not produce viable seed where winter temperatures reach 0°C or below, and is thus theorized to lose its invasive capacity in latitudes north of the Florida pan-handle. Napiergrass has been noted as being the fastest growing plant in the world (Karlsson and Vasil 1986) with possible dry biomass yields in excess of 45 Mg $ha^{-1}$ in a full season's growth (Woodard et al. 1991). Napiergrass also has an extremely efficient photosynthetic rate. This is accomplished by unique spatial and physical properties of the bundle sheath cell wall in napiergrass that allow for minimal $CO_2$ diffusion, or leakage, therefore having a higher $CO_2$ saturation at rubisco than other $C_4$ plants. Napiergrass has a bundle sheath cell wall thickness of 0.36 ± 0.05 μm compared to corn (0.2 ± 0.07 μm) and *Amaranthus* spp. (0.13 ± 0.05 μm) (von Caemmerer and Furbank 2003).

Several herbicides could potentially control napiergrass. University of Florida extension recommends a tank mix of glyphosate and imazapyr for control in natural areas (Langeland and Stoker 2001). Glyphosate is non-selective, foliar applied, and is extremely effective on perennial

grass species (Brown et al. 1988; Parochetti et al. 1975; Prostko et al. 2006; Richard 1991). It inhibits amino acid production synthesis on the shikimic pathway (Amrhein et al. 1980). Imazapic, an imidazolinone herbicide like imazapyr, inhibits enzyme production by binding to acetolactate synthase (ALS) on the branched-chain amino acid biosynthetic pathway (Saari et al. 1994; Shaner et al. 1984; Zhao et al. 1999). It is used to control broadleaf weeds, yellow nutsedge, as well as many annual and perennial grass species in peanuts (Burke et al. 2004; Grey et al. 2003; Jordan and York 2002; Webster et al. 1997) and for weed control during establishment of legumes and wildflowers in grasslands (Beran et al. 1999A; 1999B). Hexazinone causes the inhibition of photosynthesis by blocking electron transport in the photosystem II complex (Shimabukuro and Swanson 1969). Data indicates that hexazinone reduces cumulative yields in bermudagrass (Wilder et al. 2008) and reduce overall bahiagrass biomass accumulation (Sellers et al. 2008).

Napiergrass is considered a noxious weed in sugarcane production and an invasive weed to natural areas in south Florida (Florida Exotic Pest Plant Council 2005). There are no registered herbicides for selective control of napiergrass in sugarcane; therefore napiergrass needs to be controlled by spot treatments of non-selective herbicides (Rainbolt 2005). Research indicates that spot treatments of glyphosate can be effective in controlling napiergrass in established crops (Rainbolt 2005). However, little information exists about other methods of herbicidal control. Therefore, research was conducted to use the plant's unique physiological fitness to evaluate the effectiveness of several herbicides to reduce net $CO_2$ assimilation and stomatal conductance. These data could be used to evaluate herbicides effectiveness at reducing napiergrass growth and the potential for providing control of the species.

**Materials and Methods**

Nodal cuttings of 'Merkeron 534' napiergrass gathered from field stands were grown in 6 L pots[21] filled with Tifton loamy sand (87% sand, 7% silt, 6% clay; fine-loamy, kaolinitic, thermic, Plinthic Kandiudult) with pH of 6.3 and 0.8% organic matter. Stems were placed vertically in pots to allow two subsoil nodes and one above soil node. Plants were grown for approximately two months in a greenhouse at the Coastal Plains Experiment Station in Tifton, GA. All plants were weekly fertilized with 5-10-15 granular fertilizer[22] at 56 kg $ha^{-1}$ and watered as needed. Fertilizer applications ceased when treatments were initiated. Plants were trimmed to approximately five centimeters two weeks prior to herbicide treatment allowing for plant uniformity at treatment. Studies were conducted from August to October 2009. No supplemental light was provided, and cooling pads kept the temperature in the greenhouse below 32°C during the day while heaters kept nightly temperatures above 21°C.

Hexazinone, glyphosate, and imazapic[23] were applied at 200, 870, and 70 g ai $ha^{-1}$, respectively, and included a non-treated control. Crop oil concentrate (0.25% v/v) was added to the hexazinone and imazapic treatments. Treatments were applied with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$. To determine the effect of these selected herbicides on the growth reduction of napiergrass, $CO_2$ assimilation measurements were recorded with a LI-6400 by LI-COR, Inc.[24]. The LI-6400 has an open flow gas exchange system that measures photosynthesis based on differences in $CO_2$ in an air stream that is flowing to the leaf. Measurements were taken according to methods described in Ferrell et al. (2003). Leaves selected for width uniformity were measured from each plant 1 day prior to treatments, with repeated measures taken 1, 2, 3, 5, 7, 9, and 12 days after treatments (DAT) for hexazinone and glyphosate. Leaf measurements for imazapic continued to 17 and 22 DAT, or when $CO_2$

assimilation reached a constant low rate. Stomatal conductance was measured simultaneously within the leaf chamber. Constants held within the leaf chamber included an air flow rate of 500 μmol $s^{-1}$, supplied photosynthetic radiation of 1,200 μmol $m^{-2}$ $s^{-1}$ from diodes emitting red and blue light, and a $CO_2$ concentration of 400 μmol $mol^{-1}$ delivered by the system's $CO_2$ injector. Temperature within the leaf chamber was monitored but not controlled. Once the gas exchange chamber was sealed around the selected leaf, time was allowed for gas-exchange parameters to stabilize which allowed for stable $CO_2$ exchange and stomatal conductance rates before measurements were taken. Data were collected within 2 hours of solar noon (Ferrell et al. 2003).

**Experimental Design**

The experiment was a completely randomized design with four replications of treatments with the study repeated three times. The experiment was conducted three times in time. Carbon dioxide assimilation ($A_N$) and stomatal conductance ($g_s$) data were analyzed separately using the PROC NLIN procedure in SAS (SAS Inst. 2001). Data were subjected to non linear regression and responses were described by the exponential decay equation

$$y = B_0 e^{-B_1(x)} \qquad [1]$$

where $y$ is the response variable of treatment, $B_0$ is the value of the response variable ($y$) when $X$ is equal to zero, $B_1$ is the rate of decline of $CO_2$ assimilation ($A_N$) and stomatal conductance ($g_s$), and $X$ is time (DAT). Data for $CO_2$ assimilation ($A_N$)and stomatal conductance ($g_s$) for herbicide treatments were analyzed by ANOVA under the general linear models procedure and used mean separation of 95% asymptotic confidence intervals (Grey et al. 2007; SAS Inst. 2001). Data were subjected to a time to 50% growth reduction. The response was fit to the log-logistic curve

$$y = C + \left[ \frac{D - C}{1 + \left( \frac{X}{GR_{50}} \right)^{b}} \right] \quad [2]$$

where C is the lower limit, D is the upper limit, b is the slope, and $GR_{50}$ is time for 50% reduction in plant growth (Seefeldt 1995). Graphing software[25] was used for presentation of graphs.

**Results and Discussion**

There was no detectable treatment by experiment interaction, so data were combined across experiments. Data analysis indicated that there were significant differences among all treatments (Table 4.1). Rates of decline were calculated by nonlinear regression of the herbicide treatment with respect to time. Across experiments, measurement differences occurred caused by day to day climatic variations. The $GR_{50}$ for each treatment was calculated as time to 50% reduction of plant growth (Table 4.1). As stated by Ferrell et al. (2003), stomatal conductance ($g_s$) and $CO_2$ assimilation ($A_N$) are interchangeable for measure of growth reduction. Results for hexazinone are not applicable because of the rapid drop in stomatal conductance ($g_s$) that most likely took place within hours after treatment, prior to when first measurements were recorded (Data not shown).

A sharp decline in $CO_2$ assimilation ($A_N$) and stomatal conductance ($g_s$) occurred for hexazinone treated napiergrass, with $CO_2$ assimilation reaching zero by day 2 (Figure 4.1A). It is estimated the $GR_{50}$ most likely was within a few hours after treatment, and hence was not recorded. This is contradictory to field tolerance studies with hexazinone on bahiagrass and bermudagrass, other $C_4$ perennial *Poaceae* species, in that they were reported to have survived

POST treatment of hexazinone (Griffin et al. 1988; Sellers et al. 2008). In sugarcane, hexazinone is converted to several metabolites; the hydroxylated-demethylated derivative is most likely the cause of tolerance in sugarcane (Reiser et al. 1982; Vencill 2002) and similar $C_4$ species.

Carbon dioxide assimilation ($A_N$) parameters for glyphosate treated napiergrass reached zero by day 12 (Figure 4.1B). There were also visual symptoms of plant death (Data not shown). Similar reductions of $CO_2$ assimilation have been reported for glyphosate treated yellow nutsedge (Ferrell et al. 2004). The rate of decline for glyphosate treated napiergrass with respect to $CO_2$ assimilation and stomatal conductance was similar to literature on metabolism of glyphosate in $C_4$ plants. For research conducted by Hetherington et al. (1999) with non-transgenic corn, data indicated that POST applied glyphosate was not translocated but remained in leaves, which subsequently became necrotic. Data reported by Feng et al. (2003) indicates that the foliar to root ratio of glyphosate in corn was approximately two, causing a lack of injury to root biomass and possibly allowing the plants to recover. This lack of glyphosate mobility coincides with the $GR_{50}$ of 1.9 DAT (Table 4.1) indicating plant death to be prolonged through continued growth in other parts of the plant. Data indicates that glyphosate effectively controls johnsongrass (*Sorghum halpense*) (Connell and Derting 1973; Crawford and Rogers 1973; Klosterboer 1973). Research also indicates that glyphosate is readily absorbed in younger johnsongrass plants (Camacho and Moshier 1991) and that mature johnsongrass plants are more susceptible to POST glyphosate applications than immature plants (Derting et al. 1973; Klosterboer 1973; Worsham 1972.). This may lend to glyphosate being less efficacious in this study because the napiergrass plant tissue was approximately two weeks old at the time of treatment.

Imazapic treated napiergrass $CO_2$ assimilation and stomatal conductance declined in time, but, unlike hexazinone and glyphosate, did not completely halt during this study (Figure 4.1C). Measurements were continued to 22 DAT, or when parameters appeared to be at consistently low rates. The $GR_{50}$ for imazapic was 2.9 DAT (Table 4.1), longer than hexazinone and glyphosate. The gradual rate of decline for imazapic could be due to metabolism. Corn readily absorbs and metabolizes IMI herbicides, although not at an accelerated enough rate to be tolerant (Barrett 1988). Similar effects were reported in data where imazapic caused significant reduction in St. Augustinegrass (*Stenotaphrum secundatum*) stolon growth and re-growth after imazapic applications (McCarty et al. 2004). Similar trends in reduction of $CO_2$ assimilation have been reported for imazapic treated goosegrass (*Eleusine indica*). A gradual reduction in goosegrass growth occurred with continued $CO_2$ assimilation up to 6 DAT (Burke and Wilcut 2003).

**Conclusions**

Results from these greenhouse experiments indicate hexazinone, glyphosate, and imazapic reduced net $CO_2$ assimilation and stomatal conductance in napiergrass. Hexazinone and glyphosate caused napiergrass death in this study. These findings demonstrate that multiple mode of actions have the potential to effectively reduce napiergrass growth, and possibly control this potential weedy species. Management of napiergrass as a weedy species is critical for sugarcane production. The interest in napiergrass as a renewable cellulosic bio-feedstock for energy production also raises issues concerning potential methods to control escaped plants and volunteer plants in successive crops in a rotation.

Results from these experiments may not be applicable in a field scenario. As previously stated, sugarcane (morphologically similar to napiergrass) is tolerant to hexazinone which is registered for selective weed control in the crop (Anonymous 2008). Personal observations indicate that napiergrass fully recovers from hexazinone injury in the field (Data not shown). Tolerance studies conducted in sugarcane by Richard (1991) failed to achieve plant death with high rates of glyphosate. Even with 87% foliar necrosis, below ground buds began to emerge later in the season. Differences in field and greenhouse observations could be due to herbicide treatments being metabolized through the entirety of the pot-bound plants. Plants may be able to re-grow in the field from the large amounts of rhizomous material not affected by foliar herbicide applications. Variable results in studies by Dissanayake et al. (1998) indicate that glyphosate treatments increased number of sugarcane shoots in some instances. Further field studies are needed to determine levels of control that can be achieved with these and other modes of action. Effective control outside a greenhouse setting still needs to be demonstrated.

Table 4.1. Rate of decline for $CO_2$ assimilation ($A_N$) and growth reduction ($GR_{50}$) for herbicide treated napiergrass[a]

| | Rate of Decline[b] | | | | | | $GR_{50}$[d] |
|---|---|---|---|---|---|---|---|
| Herbicide | $A_N$[e] | | 95% CL | $B_0$ | | 95% CL | Days |
| Hexazinone | 3.62 | a | ±1.06 | 13.77 | a | ±0.39 | -- |
| Glyphosate | 0.34 | b | ±0.05 | 15.02 | a | ±1.17 | 1.9 |
| Imazapic | 0.13 | c | ±0.02 | 15.01 | a | ±0.75 | 2.9 |
| Non-treated | 0.03 | d | ±0.01 | 14.69 | a | ±0.68 | -- |

[a]Each herbicide for first-order rate constants for each column followed by the same letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$). General linear models procedures were used for mean separation with 95% asymptotic confidence intervals.

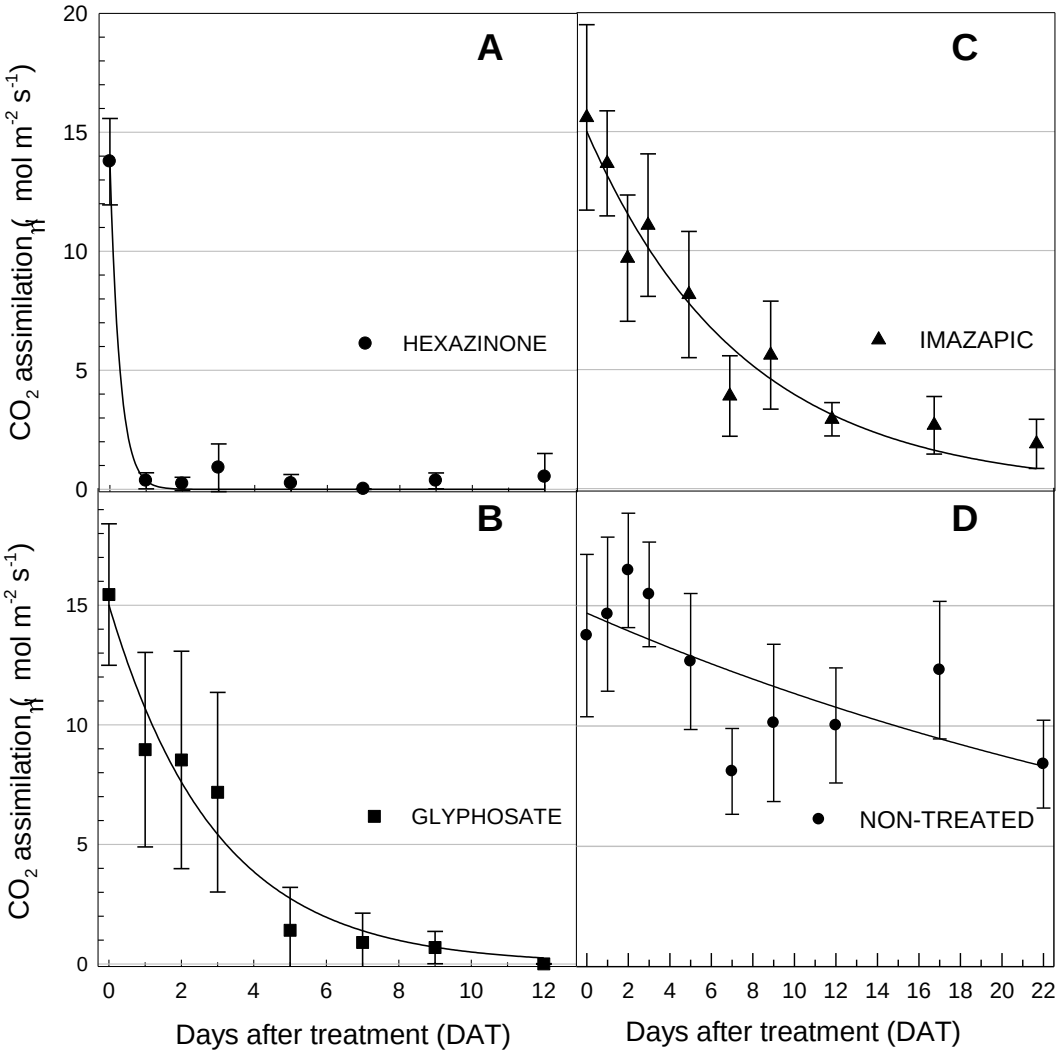
[b]Rates of decline were calculated by nonlinear regression of the herbicide treatments with respect to time (0-12 DAT for hexazinone and glyphosate and 0-22 DAT for imazapic and non-treated).

[c]Abbreviations: $A_N$, rate of decline of $CO_2$ in μmol $m^{-2}$ $s^{-1}$; CL, confidence limit..

[d]Values representative of time to 50% reduction of growth in days for stomatal conductance. Data not available for hexazinone due to values being reached prior to 1 DAT.

[e]$A_N$ is representative of $B_1$ in the equation from Figure 4.1.

*Figure 4.1*. Carbon assimilation ($A_N$) for herbicide treated napiergrass measured by Li-Cor 6400 for hexazinone (A), glyphosate (B), imazapic (C), and non-treated (D). Tests were conducted three times. Error bars represent standard error about the mean. Data were subjected to non linear regression and responses were described by the exponential decay equation $y = B_0 e^{-B_1(x)}$ where $y$ is the response variable of treatment, $B_0$ is the value of the response variable ($y$) when $X$ is equal to zero, $B_1$ is the rate of decline of $A_N$ and $X$ is time (DAT). Parameter response variables for $B_1$ are in Table 4.1.


A
B
C
D
HEXAZINONE
GLYPHOSATE
IMAZAPIC
NON-TREATED
CO2 assimilation (µ mol m-2 s-1)
Days after treatment (DAT)
Days after treatment (DAT)

# CHAPTER 5

## HERBICIDE EFFECT ON NAPIERGRASS (*PENNISETUM PURPUREUM SHUM.)* CONTROL: FIELD TRIALS[3]

[3] Cutts, G.S., T.L. Grey, R.D. Lee, T.M. Webster and W.K. Vencill. To be submitted to Weed Science.

**Abstract:** Below 30 degrees latitude in the United States, napiergrass is considered a noxious weedy species. It is also an invasive weed in Florida as well as in tropical regions in Southeast Asia, throughout the Pacific, and South Africa. Field studies were initiated in October of 2008 with the napiergrass cultivar 'Merkeron 534' to evaluate herbicides with different tank mixtures and application dates for napiergrass control at Tifton and Ty Ty, Georgia. Autumn treatments of glyphosate, glyphosate plus sethoxydim, hexazinone, and imazapic were applied in September 2009. A spade tillage treatment was made in January 2010. Spring treatments of glyphosate, glyphosate plus sethoxydim, and imazapyr were applied in April 2010 with split applications of autumn treatments. Autumn treated napiergrass stunting and chlorosis were 99% and 98%, respectively, for glyphosate, and 96% and 86%, respectively, for glyphosate plus sethoxydim by 50 DAA.

**Introduction**

In the lower latitudes of the tropical and sub-tropical United States, napiergrass is considered a noxious weedy species. Napiergrass is also an invasive weed in tropical regions and has been listed on the invasive species list by the Florida Exotic Pest Plant Council (Florida Exotic Pest Plant Council 2005). However, napiergrass is described as having latitudinal restrictions to its invasive capacity above 30 degrees latitude north and south. For example, in north Florida and southern Georgia cooler temperatures restrict its invasive potential by inducing winter dormancy. Frost kills above ground foliage, though soil must be frozen to kill below ground rhizomes (Duke 1983). The species also only flowers under short days and will not produce viable seed where winter temperatures reach 0°C or below, thus losing its invasive capacity in latitudes north of the Florida pan-handle (Hanna and Ruter 2005).

The species was introduced to the United States as a forage crop, though it is no longer used in this capacity and it has become a major weed in natural areas and sugarcane production. The problem is further exacerbated when areas once established with napiergrass for forage are cultivated for other crops and vegetative material is unintentionally dispersed by mechanical soil preparation.

Napiergrass is especially difficult to selectively control in sugarcane because of the morphological similarities between the two species. Cultural control, such as tillage or hand removal of vegetative material, can be an inexpensive way of removing napiergrass stands in ditches and field borders around sugarcane fields. However, mechanical control can often result in larger infestations by disturbing plant parts. Plants should be totally removed because of their vegetative reproductive characteristics (Rainbolt 2005).

One of the more effective methods for controlling napiergrass is repeated, timely applications of glyphosate. Glyphosate is non-selective, foliar applied, and is extremely effective on perennial grass species (Brown et al. 1988; Parochetti et al. 1975; Prostko et al. 2006; Richard 1991). Glyphosate inhibits amino acid production synthesis on the shikimic pathway (Amrhein et al. 1980). Glyphosate is recommended at a 2% a.e. (v/v) solution rate for control of napiergrass (Rainbolt 2005). However, *in vitro* tests on the embryogenic callus of napiergrass with low rates of glyphosate ($\leq 0.005$ g a.e. $L^{-1}$ solution) were not inhibitive of callus formation (Rajasekaran et al. 1986). Variable results in studies by Dissanayake et al. (1998) reported that glyphosate increased number of sugarcane shoots in some instances. There has been indication that death of apical meristems from glyphosate treatments causes an increase in shoot production from lateral buds in sugarcane stems where sugar reserves can still feed development (Richard 1991).

Other herbicides used to control grasses could be effective for napiergrass control. Imazapic and imazapyr, both IMI herbicides, inhibit enzyme production by binding to ALS on the branched-chain amino acid biosynthetic pathway (Saari et al. 1994; Shaner et al. 1984; Zhao et al. 1999). Imazapic is used for grass control in peanuts (Burke et al. 2004; Jordan and York 2002) and for weed control during establishment of legumes and wildflowers in grasslands (Beran et al. 1999A; 1999B). Data from Webster et al. (1997) indicates that imazapic controls many broadleaf weeds and yellow nutsedge as well. Imazapyr is used in tank mixes with glyphosate or alone to control cogongrass, an invasive perennial grass in the southeastern United States (Faircloth 2005).

Hexazinone causes the inhibition of photosynthesis by blocking electron transport in the photosystem II complex (Shimabukuro and Swanson 1969). Previous research indicates that

hexazinone reduces cumulative yields in bermudagrass (Wilder et al. 2008) and reduces overall bahiagrass biomass accumulation (Sellers et al. 2008). These grasses are also $C_4$ perennial species, and therefore hexazinone could have similar inhibitory effects on napiergrass.

The potential invasiveness of napiergrass either as a plant that escapes cultivation or one that persists in subsequent crop rotation is a concern when considering its potential production as a biofuel feedstock. A similar potential bio-energy crop species, *Miscanthus* spp. (*M. sinesis* and *M. sacchariflorus*) have escaped in Ohio and Indiana and caused local concern (Lewandowski et al. 2000). An executive order on invasive species stated that it is necessary to determine whether there will be any potential harm from new biofuel crops as invasive species, and measures need to be taken to minimize the risk of harm before United States federal funds can be used to develop them (White House 1999). The potential benefits of napiergrass as a viable feedstock for biofuel are great enough to move forward with production in Georgia, but the ability to effectively control this $C_4$ perennial *Poaceae* species is crucial to understand how and where it can be planted and utilized as a biofuel crop. Fully established, napiergrass is difficult to control due to its rapid growth habit. There is limited information present about control of established napiergrass stands. Therefore, herbicides that are effective in controlling perennial *Poaceae* species were evaluated with different tank mixtures and application dates in order to evaluate effectiveness of napiergrass control.

## Materials and Methods

Field studies for napiergrass control were initiated in October 2008, planting the cultivar ‘Merkeron 534’. Planting stock was taken from an established napiergrass stand at the Coastal Plains Experiment Station in Tifton, Georgia. Stems were cut from the bottom meter of the plant

that contained 5 to 10 viable nodes. Planting sites were located at Tifton and Ty Ty, Georgia. The soil type at these locations was a Tifton loamy sand (fine-loamy, siliceous, thermic plinthic Kandiudults) with a pH of 6.0 to 6.2 and 0.5 to 0.8% organic matter. Plots were in bedded, conventionally tilled fields. A trenching implement was used to plow a single planting row to a 10 cm depth through the center of plots that were 1.8 meters wide by 6 meters long. Stems were then laid horizontally, end to end, in the planting trench, covered by hand and rotor-packed for adequate soil contact with vegetative material. Plants emerged two to three weeks after planting. The first freeze occurred on November 19, 2008 at both locations. Plots were fallow for the winter of 2008/2009.

Napiergrass emergence in the spring of 2009 was inconsistent throughout plots. Plants were allowed to grow untreated for eleven months. In September of 2009, all plots at both sites were mowed down using a rotary mower to a height of approximately 7 cm. Because of inconsistent emergence and plant size within plots, relative plant size within plots was quantified by measuring the area of the stem "clumps" present. Treatments were then blocked by clump area.

Prior to autumn treatments, desiccated biomass left from mowing was raked away from clumps to allow herbicide contact with newly emerged foliar re-growth. Treatments (Table 5.1) were applied on September 16, 2009 (Ty Ty) and September 22, 2009 (Tifton) with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$. The first freeze of 2009 occurred on December 6 for both locations, after which all surviving and non-treated plants began to senesce. After induced to winter dormancy, all plants were mown to 7 cm height on January 14, 2010. A winter spade tillage treatment was implemented on January 19, 2010 at both locations using a power-spader[26]. This was a power-driven primary-tillage implement that tills a swath 1.8 meters wide at a depth of 36 cm. The spader operates off a tractor PTO at 540 revolutions per minute

(rpm) and the gear box reduces the speed down to 60 rpm. A bed finishing device attached to the rear of the power-spader simultaneously smoothed the tilled bed. It became apparent at the end of winter dormancy (March 12, 2010) that the winter spade tillage alone would likely be ineffective in controlling napiergrass due to the amount of plant material undisturbed and amount of napiergrass re-growth. Also, observations before winter dormancy showed all autumn treated plants had foliar re-growth (Data not shown). For these reasons, spring tillage treatments originally planned were replaced with a spring application of imazapyr[27]. All other autumn treated napiergrass received a split application of their original treatments. Rates of glyphosate were doubled from autumn application rates.

On April 1, 2010, spring treatments (Table 5.2) were made with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$. Spring treatments included imazapyr, glyphosate, glyphosate plus sethoxydim, and split applications of all autumn treatments.

Field observations for autumn treatments included plant stunting and injury data made at 7, 15, 21, and 50 days after application (DAA) as compared to the non-treated control. Field observations for spring treatments included injury data at 10 DAA.

**Experimental Design**

Treatments were organized into a randomized block design according to quantified clump area with 4 replications of treatments. Data for autumn and spring treatments were analyzed separately and subjected to analysis of variance (SAS Inst. 2001). Treatment means were separated using Fisher's Protected LSD ($P \leq 0.05$).

**Results and Discussion**

For autumn treatment results, data were combined over location and replication for presentation. There was no correlation between quantified clump size and treatment effect for any autumn treatment. A trend did not exist when clump size and injury data were plotted against one another (Data not shown). Data analysis indicated differences among treatments. At 15 DAA, severe stunting and chlorosis was induced by glyphosate (87% and 84% respectively) as well as glyphosate plus sethoxydim treated napiergrass (86% and 85% respectively). Stunting and chlorosis increased with both treatments (99.4% and 98.3%, respectively, for glyphosate; 95.6% and 85.6%, respectively, for glyphosate plus sethoxydim) by 50 DAA (Table 5.2). Results in this study are similar to findings that glyphosate plus sethoxydim has 78% control of volunteer corn (Beckett et al. 1992; Soltani et al. 2005; Young and Hart 1997).

Hexazinone induced minimal napiergrass chlorosis (22%) 15 DAA, and injury was transient and was not observed 50 DAA. However, in other $C_4$ perennial *Poaceae* species, such as bermudagrass and bahiagrass, research indicates that hexazinone reduces cumulative yields and overall biomass accumulation (Sellers et al. 2008; Wilder et al. 2008). But field tolerance studies with hexazinone on bahiagrass and bermudagrass indicate the species survived POST treatment applications (Griffin et al. 1988; Sellers et al. 2008). In sugarcane, hexazinone is converted to several metabolites including a hydroxylated derivative, a hydroxylated-dementhylated derivative, and a hydroxylated-deaminated derivative. The hydroxylated-demethylated derivative is most likely the cause of tolerance in sugarcane (Reiser et al. 1982; Vencill 2002) and similar $C_4$ species such as napiergrass.

Non-treated napiergrass plants survived cold temperatures during the winter of 2009 and 2010. There was a 14 day period in January 2010 of nightly low temperatures below freezing

(Figure 5.1). Although sub-freezing air temperatures kill above ground napiergrass foliage, soil temperatures also must be below 0°C to kill sub-soil biomass (Duke 1983). Soil temperatures at 2 inches never were below freezing in January 2010 (Figure 5.2) and plants were able to survive.

At 10 days after spring application, data analysis indicated that there were differences among treatments (Table 5.4). Highest levels of napiergrass injury occurred with autumn and spring glyphosate (99% and 96% at Tifton and Ty Ty, respectively) and glyphosate plus sethoxydim (99% and 93% at Tifton and Ty Ty respectively). Field observations indicated plant death (Data not shown). Research indicates that glyphosate applied before winter dormancy has effective spring control of johnsongrass (Connell and Derting 1973; Crawford and Rogers 1973; Klosterboer 1973), a similar rhizomous $C_4$ perennial *Poaceae* species. Spade tillage treated napiergrass had no injury compared to the non-treated. Data indicated high levels of injury (70% and 83% at Tifton and Ty Ty, respectively) on spring imazapyr treated napiergrass, but has yet to be determined whether plant death will follow.

**Conclusions**

Glyphosate provided the highest levels of napiergrass control. Autumn applications alone however did not consistently result in complete plant death. Repeated applications in autumn and spring are needed to effectively control the plant, as well as subsequent applications to ensure all vegetative material that might be dispersed is also killed. Further control scenarios need to be studied before large scale production of napiergrass for a biofuel feedstock can begin in Georgia.

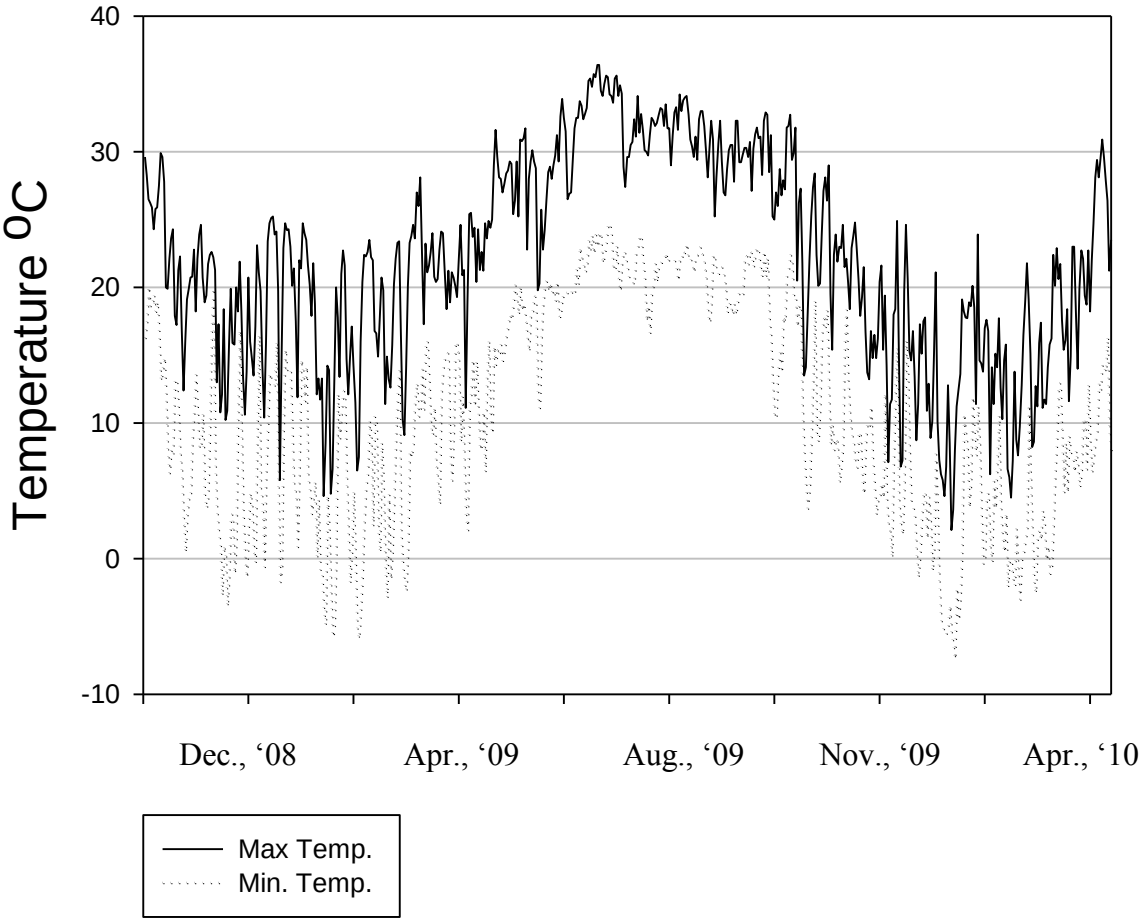



*Figure 5.1*. Maximum and minimum air temperature for Tifton and Ty and Ty, Georgia. Temperature presented in degrees Celsius over the duration of the field control study, from October 6, 2008 to April 11, 2010.

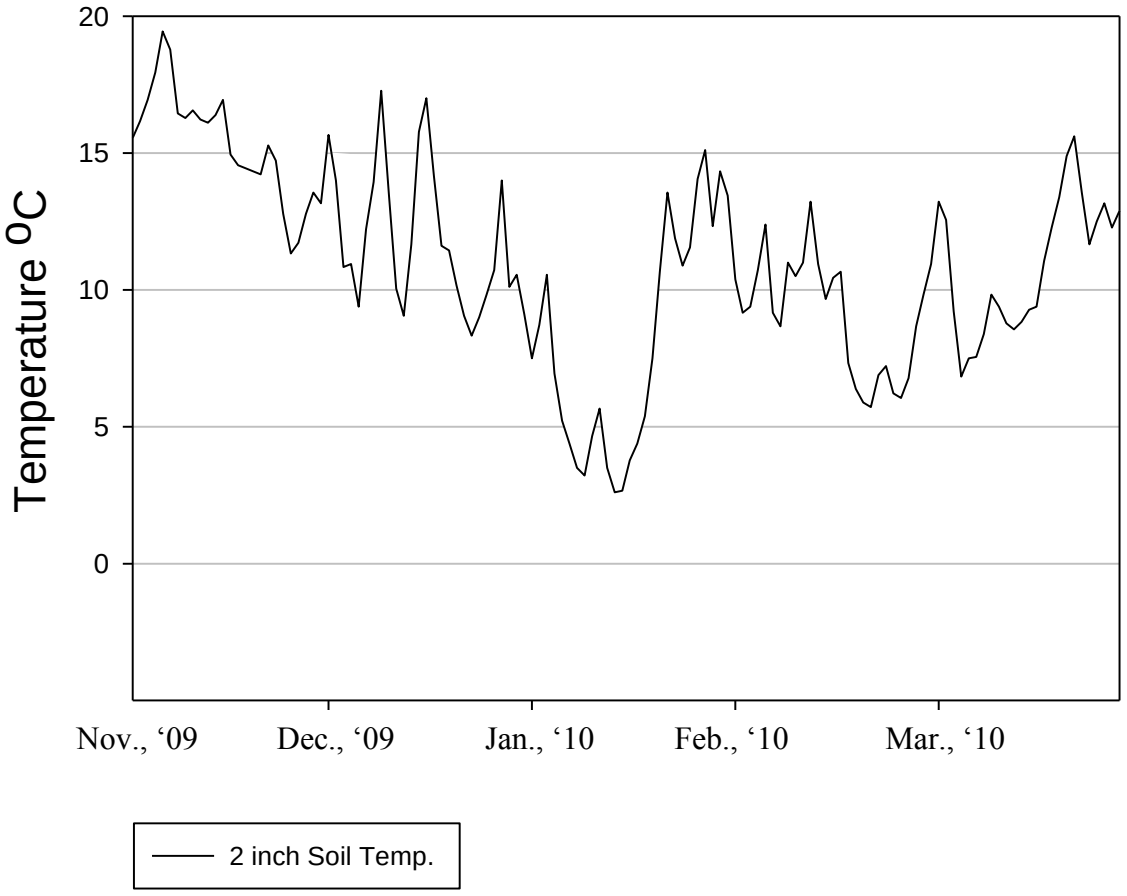



*Figure 5.2*. Soil temperature at 2 inches for Tifton and Ty and Ty, Georgia. Temperature presented in degrees Celsius from November 6, 2009 to March 17, 2010.

Table 5.1. Autumn treatments for napiergrass control at Tifton and Ty Ty, GA in 2009[a]

| Treatments | Rate |
|---|---|
| | kg ai $ha^{-1}$ |
| Non-treated | -- |
| glyphosate | 0.84 |
| imazapic plus COC | 0.07 + 0.25% (v/v) |
| Glyphosate plus sethoxydim plus COC | 0.84+0.43 + 0.25% (v/v) |
| Hexazinone plus COC | 0.20 + 0.25% (v/v) |

[a]All treatments applied with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$ on September 16, 2009 at Ty Ty and September 22, 2009 at Tifton.

Table 5.2. Napiergrass injury from autumn treatments prior to dormancy at Tifton and Ty Ty, GA in 2009[a]

| Treatments | Stunting | | | | Chlorosis | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 DAA | | 50 DAA | | 15 DAA | | 50 DAA | |
| | % | | | | % | | | |
| glyphosate | 87 | a | 99 | a | 84 | a | 98 | a |
| imazapic plus COC | 64 | b | 84 | b | 54 | b | 75 | b |
| glyphosate plus sethoxydim plus COC | 86 | a | 95 | a | 85 | a | 85 | ab |
| hexazinone plus COC | 51 | b | 29 | c | 23 | c | 0 | c |

[a]All treatments applied with a $CO_2$ pressurized backpack sprayer delivering 140 L $ha^{-1}$ on September 16, 2009 at Ty Ty and September 22, 2009 at Tifton.

[b]Values followed by the same letter for each column do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

Table 5.3. Winter and spring treatments for napiergrass control at Tifton and Ty Ty, GA in 2009/2010[a]

| Treatments | Rate |
|---|---|
| | kg ai $ha^{-1}$ |
| Autumn non-treated | -- |
| glyphosate[b] | 0.84 |
| imazapic plus COC[b] | 0.07 + 0.25% (v/v) |
| glyphosate plus sethoxydim plus COC[b] | 0.84+0.43+0.25%(v/v) |
| hexazinone plus COC[b] | 0.20 + 0.25%(v/v) |
| spade tillage[c] plus glyphosate[d] | -- |
| imazapyr[d] | 2.78 + 0.25% (v/v) |
| glyphosate[d] | 1.68 |
| glyphosate plus sethoxydim plus COC[d] | 1.68+0.43+0.25%(v/v) |
| Spring non-treated[d] | -- |

[a]Establishment experiments located in Plains, GA, and Ft. Valley, GA.

[b]Fall treatments received a split application on April 1, 2010.

[c]Implemented on January, 19 2010.

[d]Applied on April 1, 2010.

Table 5.4. Napiergrass injury from autumn and spring treatments 10 DAA[a]

| Treatments | Chlorosis | | | |
|---|---|---|---|---|
| | % | | | |
| | Tifton | | Ty Ty | |
| Autumn non-treated | 0 | e | 0 | d |
| glyphosate[b] | 99 | a | 96 | a |
| imazapic plus COC[b] | 81 | b | 66 | c |
| glyphosate plus sethoxydim plus COC[b] | 99 | a | 83 | ab |
| hexazinone plus COC[b] | 4 | de | 14 | d |
| spade tillage[c] plus glyphosate[d] | 0 | e | 0 | d |
| imazapyr[d] | 70 | bc | 83 | b |
| glyphosate[d] | 51 | c | 55 | c |
| glyphosate plus sethoxydim plus COC[d] | 67 | c | 50 | c |
| Spring non-treated[d] | 0 | e | 0 | d |

[a]Establishment experiments located in Plains, GA, and Ft. Valley, GA.

[b]Fall treatments received a split application on April 1, 2010.

[c]Implemented on January, 19 2010.

[d]Applied on April 1, 2010.

[e]Values followed by the same letter for each column do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

# CHAPTER 6

# SUMMARY AND CONCLUSIONS

For establishment of napiergrass, pendimethalin plus atrazine allowed for consistently higher weed control, stem height, and dry biomass yields when used PRE. A continuation of this study is needed before herbicides for establishment in napiergrass can be recommended. POST weed control was also necessary during this study. Observations made with subsequent POST applications show strong potential for excellent weed control and crop tolerance to napiergrass. Further research is needed to explore these and other post-emergent weed control options.

Results from preliminary greenhouse experiments indicated that hexazinone, glyphosate, and imazapic all reduced net $CO_2$ assimilation and stomatal conductance in napiergrass. These findings demonstrate that multiple modes of action have the potential to effectively reduce napiergrass growth, and possibly control this weedy species. Results from these experiments may not be applicable in a field scenario. Plants could re-grow in the field from the large amounts of sub-soil rhizomous material not affected by foliar herbicide applications. Further field studies are needed to determine levels of control that can be achieved with these and other modes of action. Effective control outside a greenhouse setting still needs to be demonstrated.

In field control studies, non-treated napiergrass plants were able to survive prolonged periods of nightly sub-freezing temperatures in the winter of 2009 and 2010. Glyphosate provided the highest levels of napiergrass control. Repeated applications in autumn and spring are needed to

effectively control the plant, as well as subsequent applications to ensure all vegetative material that might be dispersed is also killed.

The potential benefits of napiergrass as a viable feedstock for biofuel are great enough to move forward with production in Georgia. However, steps need to be taken to study further agronomic variables, determine the potential for napiergrass to become invasive in Georgia, and develop effective control methods for crop rotation during production and prevention of escape.

## SOURCES OF MATERIALS

[1]Roundup Weather Max®, Monsanto Company, St. Louis, MO 63167.

[2]Atrazine 4L®, Helena Chemical Company, Collierville, TN 38017

3Crop oil concentrate; Chem Nut, Inc., Leesburg, GA 31763

[4]Command 3ME®, FMC Corporation, Philadelphia, PA 19103

[5]Di-Syston 8®, Bayer CropScience LP, Research Triangle Park, NC 27709

[6]Direx 4L®, E.I. du Pont de Nemours and Company, Wilmington, DE 19898

[7]Non-ionic surfactant; Chem Nut, Inc., Leesburg, GA 31763

[8]Prowl $H_2O$®, BASF Corporation, Research Triangle Park, NC 27709

[9]Sencor 4®, Bayer CropScience LP, Research Triangle Park, NC 27709

[10]Spartan 4F®, FMC Corporation, Philadelphia, PA 19103

[11]Valor®, Valent U.S.A. Corporation, Walnut Creek, CA 94596

[12]Evik 80 W®, Syngenta Crop Protection Inc., Greensboro, NC 27409

[13]Dual II Magnum®, Syngenta Crop Protection Inc., Greensboro, NC 27409

[14]Callisto®, Syngenta Crop Protection Inc., Greensboro, NC 27409

[15]Laudis®, Bayer CropScience LP, Research Triangle Park, NC 27709

[16]Velpar L®, E.I du Pont de Nemours and Company, Wilmington, DE 19898

[17]Sinbar®, Teesenderlo Kerely Inc., Phoenix, AZ 85008

[18]2,4-D Amine 4®, Helena Chemical Company, Collierville, TN 38017

[19]Basagran®, BASF Corporation, Research Triangle Park, NC 27709

[20]Poast®, BASF Corporation, Research Triangle Park, NC 27709

[21]Nursery Supplies, Inc., Chambersburg, PA 17201

[22]Agrium U.S. Inc., Denver, CO 80237

[23]Cadre®, BASF Corporation, Research Triangle Park, NC 27709

[24]LI-6400, LI-COR Inc., Lincoln, NE 68504

[25]SigmaPlot® for Windows version 10.0, Systat Software, Inc., Richmond, CA 94804.

[26] Imants power-spader, Autrusa, Inc. Perkiomenville, PA 18074

[27]Arsenal®, BASF Corporation, Research Triangle Park, NC 27709

[28]Beyond®, BASF Corporation, Research Triangle Park, NC 27709

[29]Osprey®, Bayer CropScience LP, Research Triangle Park, NC 27709

[30]Hoelon®, Bayer CropScience LP, Research Triangle Park, NC 27709

[31]Finesse®, E.I. du Pont de Nemours and Company, Wilmington, DE 19898

[32]Harmony®, E.I. du Pont de Nemours and Company, Wilmington, DE 19898

[33]Express®, E.I. du Pont de Nemours and Company, Wilmington, DE 19898

[34]Peak®, Syngenta Crop Protection Inc., Greensboro, NC 27409

[35]Powerflex®, Dow AgroSciences LLC, Indianapolis, IN 46268

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# APPENDIX A

## Literature Review

Winter wheat is an important fall-seeded crop throughout much of the south. It is a component of many double-crop production systems in which crops such as soybean or cotton are seeded immediately after wheat harvest (Culpepper and York 1997). Italian ryegrass (*Lolium multiflorum* L.) in wheat is a common and troublesome weed throughout this region (Webster and MacDonald 2001; Webster 2008). However, Italian ryegrass does have use in hay and pasture forage, turf, and commercial seed production that occurs in the northwestern United States. Inability to control Italian ryegrass in winter wheat can result in reduced yields and /or quality and cause double crop planting to be inefficient. Many producers often burn stubble fields in order to improve planting efficiency, but this is often seen as a growing environmental problem.

Diclofop can be used for PRE or POST control of Italian ryegrass (Justice et al. 1994; Robinson and Banks 1983; Shaw and Wesley 1991), but has either proven ineffective or has resulted in unacceptable crop injury. The second factor that contributes to control failure is diclofop-resistant Italian ryegrass. Italian ryegrass resistance to diclofop was first reported in 1987 in Oregon (Betts et al. 1992; Stranger and Appleby 1989). It has subsequently been reported in the southeastern United States (Heap 2003; Kuk et al. 2000) and throughout the world (Bravin et al. 2001; De Prado, et al. 1999; De Prado,et al. 2000; Eberlein et al. 1999; Betts et al. 1992). Grass resistance to diclofop in the United States has increased rapidly since 1990

and has been reviewed by Kuk et al. (2000). The widespread development of diclofop resistance in Italian ryegrass reduces control options in wheat and decreases the potential for double crop production systems.

A Clearfield® wheat cultivar has been developed by the University of Georgia small grains breeding program. UGA Clearfield wheat varieties are not GMOs because they were developed using traditional breeding techniques. No foreign DNA was inserted during development. This process began in 2000 using traditional backcrossing methods with yearly herbicide screenings and selections. In 2007 a cultivar was identified for release and seed increases are now occurring. Imazamox is registered for use on Clearfield wheat cultivars. Imazamox is a member of the imidazolinone (IMI) family of herbicides, along with imazapic, imazethapyr, and several other herbicides that are used in Clearfield crop production. Currently Clearfield corn, rice, canola (*Brassica campestris* L.), wheat, and sunflower (*Helianthus annuus*) are cropping systems used in the United States. However, until recently there were no Clearfield wheat cultivars adapted to the southeastern United States so the use of this technology has not been available to farmers of this region.

Clearfield crops have tolerance to IMI herbicides. While Clearfield wheat primarily is associated with the IMI family of herbicides, there are other acetolactate synthesis (ALS) inhibiting herbicides which have the potential to be used in Clearfield wheat systems. Mesosulfuron is a sulfonylurea providing excellent control of annual ryegrass in winter wheat (Bayer 2007), and is an ALS inhibiting herbicide. There have been reports of conventional wheat (Culpepper unpublished data 2007), oat (*Avena* spp.), and rye injury (MacCrae et al. 2007) that occurred after treatment with mesosulfuron. However, farmers often must use mesosulfuron as it is their only option if they have diclofop resistant Italian ryegrass. It can be POST applied

for excellent control of Italian ryegrass from the 2-leaf stage to the end of tillering of the crop, providing effective residual weed control (Crooks and York, 2002). Other ALS herbicides that are registered for Georgia wheat production include thifensulfuron, chlorsulfuron, prosulfuron, and tribenuron. These herbicides provide control of different weed spectrums, but they all have warnings about potential wheat injury (Culpepper 2007).

With the introduction of Clearfield wheat by the University of Georgia small grains breeding program, farmers in the southeastern United States will soon have the option to incorporate newer technologies into their crop production. Currently there is little information about the spectrum of weeds controlled with imazamox in wheat for this region. Additionally, other ALS herbicides may also be used in Clearfield wheat, but no information is available as to their effect on growth, development, or yield on this cultivar.

# APPENDIX B

## IMIDAZOLINONE-TOLERANT WINTER WHEAT (*TRITICUM AESTIVUM*) WEED CONTROL AND CROP RESPONSE TO ALS-HERBICIDES IN GEORGIA.[4]

[4] Cutts, G.S., T.L. Grey, R.D. Lee, T.M. Webster and W.K. Vencill. To be submitted to Weed Technology.

**Abstract:** Studies were initiated in Plains, Georgia evaluating Italian ryegrass control in imidazolinone (IMI)-tolerant wheat using imazamox, mesosulfuron, and diclofop. Treatments were applied at one- and two-times rates with variables of tank mixture and application dates. Studies were also initiated to evaluate efficacy of the acetolactate synthase (ALS) inhibiting herbicides registered for wheat weed control at one- and two-times rates in an IMI-tolerant cultivar (GA-7T) and a conventional cultivar (AGS 2000). Treatments for the weed control study were applied to GA-7T at emergence (EMERG) and Feekes stage 2 (POST). Significantly lower Italian ryegrass control (< 60%) was observed with all treatments containing only POST applications. Diclofop treatments allowed for maximum Italian ryegrass control and minimal GA-7T injury. For the efficacy study in 2007, mesosulfuron at 40 g ai $ha^{-1}$ 7 days after application (DAA) caused 17% wheat stunting and 13% by 34 DAA, but was transient and not visible at harvest. In 2008, chlorsulfuron at 60 g $ha^{-1}$ had 5% stunting at 7 DAA, but injury in all treatments was not observable by 34 DAA. AGS 2000 yield in 2007 was 2900 kg $ha^{-1}$ for mesosulfuron, with greatest yield (3280 kg $ha^{-1}$) achieved with the non-treated control. There were no differences for yield in treatments for AGS 2000 in 2008, or for GA-7T in 2007 or 2008. Treatments could be used in the future in cases of herbicide resistant weeds in winter wheat production.

INDEX WORDS: chlorsulfuron, diclofop, imazamox, IMI-wheat, Italian ryegrass mesosulfuron, prosulfuron, pyroxsulam, thifensulfuron, tolerance, tribenuron, weed control.

**Introduction**

Winter wheat is a vital part of cropping systems in the Coastal Plain of the southeastern United States. It is a fall seeded crop that is integrated in crop rotations with other row crops such as corn, cotton, peanut, and soybean. Italian ryegrass in winter wheat production can inhibit harvest and reduce yields. Diclofop has been an acceptable form of control for Italian ryegrass in wheat, but the desired effect can be variable due to application inconsistencies. Italian ryegrass resistance to diclofop has also been documented in the southeastern United States (Kuk and Burgos 2007). Wheat varieties have been developed by conventional breeding techniques for tolerance to the imidazolinone (IMI) herbicides and have been marketed under the Clearfield® brand since 2001. This herbicide family inhibits the acetolactate synthase (ALS) enzyme which is involved in the bio-synthesis of branched chain amino acids in plants. ALS herbicides not in the IMI family could have acceptable control of Italian ryegrass in Clearfield wheat systems. Therefore studies were initiated to evaluate Italian ryegrass control in IMI-tolerant winter wheat using imazamox, mesosulfuron, and diclofop. Currently Clearfield corn, rice, canola, wheat, and sunflower are cropping systems used in the United States. However, until recently there were no Clearfield wheat cultivars adapted to the southeastern United States so the use of this technology has not been available to farmers of this region.

With the introduction of Clearfield wheat by the University of Georgia small grains breeding program, farmers will soon have the option to incorporate newer technologies into their crop production. Currently there is little information about the spectrum of weeds controlled with imazamox in wheat for the southeastern United States. Additionally, other ALS herbicides may also be used in Clearfield wheat, but no information is available as to their effect on growth, development, or yield on this cultivar.

**Objectives**

In order to evaluate the use of ALS herbicides in Clearfield wheat systems, field trials that emphasize weed control, crop response, and yield as standards to measure their potential use were conducted. Two studies were initiated. The first study evaluated Italian ryegrass control in the UGA IMI-tolerant wheat cultivar GA-7T with diclofop and other ALS herbicides. Since wheat tolerance to these and other ALS herbicides is unknown, the second study evaluated efficacy of ALS herbicides labeled for wheat production at one- and two-times rates on GA-7T and a non-IMI tolerant cultivar (AGS 2000). Studies were initiated in November of 2007 and 2008, and were conducted at the University of Georgia research farm at the Southwest Research Center near Plains, Georgia.

**Materials and Methods**

Studies were initiated in November of 2007 and 2008 near Plains, Georgia. Wheat cultivars were sown into conventionally tilled beds. Plot size was 1.8 meters wide by 6 meters long. Treatments for the weed control study included imazamox[28], mesosulfuron[29], and diclofop[30] at varying concentrations and application timings (Appendix 1). At wheat crack, emergence (EMERG) treatments were applied at one- and two-times recommended rates with tank mixtures of each. Subsequent treatments were made at Feekes stage 2 (POST) at one- and two-times rates. All treatments except diclofop received a non-ionic surfactant at 0.5% v/v and UAN at 4.7 L $ha^{-1}$. Italian ryegrass was sown into the rear 2 meters of each plot in order to assess level of control. Percent control of Italian ryegrass, percent foliar wheat injury, and final yield data were collected.

Treatments for the efficacy study included chlorsulfuron[31], mesosulfuron, thifensulfuron[32], tribenuron[33], prosulfuron[34], and pyroxsulam[35] at one- and two-times rates (Appendix 2), and were applied according to their labeled timing recommendation to cultivars GA-7T and AGS 2000. Percent foliar wheat injury and final yield data were collected.

**Experimental Design**

Plots for both studies were arranged in a randomized complete block design, with four replications of treatments. Data were normally distributed and analyzed using the PROC GLM procedure (SAS Inst. 2001). Treatment means were separated using Fisher's Protected LSD ($P \leq 0.05$). For the control study results, data were not significant for year, so data for the 2007/2008 season and the 2008/2009 season were combined for presentation. For the results in the tolerance study, injury data were not significant for cultivar so were combined over cultivar and by season. Yield was separated by year and cultivar.

**Results and Discussion**

In the weed control study, significant wheat injury (> 5%) occurred with EMERG applications of mesosulfuron at 20 and 40 g ai $ha^{-1}$, and imazamox at 80 g ai $ha^{-1}$. Previous studies have also indicated 12% and 23% injury with mesosulfuron and imazamox treatments, respectively (Crooks et al. 2003; Frihauf et al. 2005). Significantly lower Italian ryegrass control (< 60%) was observed with all treatments containing only POST applications. These findings are congruent with other studies that indicate POST treatments are not effective in controlling Italian ryegrass and that there is greater activity when treatments are applied PRE (Liebl and Worsham 1987; Wilson and Hines 1997). Severe injury (21%) occurred with POST applications

of imazamox at 80 g ai $ha^{-1}$ (Appendix 3), which is similar to imazamox injury reported in studies by Frihauf et al. (2005). There were no significant differences in yield among treatments, which is similar to results from Deeds et al. (2006) where wheat was reported to recover from imazamox injury by the end of the growing season.

For the efficacy study, crop stunting and final crop yield data were collected. In 2007, mesosulfuron caused significant stunting (17%) at 40 g ai $ha^{-1}$ 7 days after application (DAA), and continued to have significant stunting (13%) through 34 DAA. Bailey et al. (2004) reported 29% stunting at 21 DAA in AGS 2000. In 2008, significant stunting (5%) occurred with chlorsulfuron at 60 g ai $ha^{-1}$ 7 DAA, but was transient and not visible at harvest. Grey and Bridges (2003) reported a reduction in wheat chlorsulfuron injury from 18% 44 DAA to 10% 151 DAA.

Yields for AGS 2000 in 2007 were significantly reduced to 2900 kg $ha^{-1}$ with mesosulfuron treatments at 40 g ai $ha^{-1}$ compared to the non-treated control. Greatest yields, 3280 kg $ha^{-1}$, were observed with chlorsulfuron treatments at 60 g ai $ha^{-1}$ and with the non-treated control. There were no significant differences in yield for AGS 2000 in 2008. GA-7T did not show significant differences in yield among treatments in 2007 or 2008.

**Conclusions**

For the weed control study, diclofop achieved the highest levels of Italian ryegrass control and allowed for minimal crop injury. However, other treatments evaluated could be used in cases of Italian ryegrass resistance to diclofop. A continuation of this study is needed to further evaluate other ALS herbicides that could be used in this capacity.

In the efficacy study, all treatments evaluated grew out of any injury sustained by mid- to late season. Use of these herbicides in winter wheat cultivars is not yet recommended, but could be used in the future in cases of herbicide resistant weeds in winter wheat production. POST applied herbicides in this study could have application in intercropping systems with wheat and peanut. Since peanuts are not yet harvested at the time of wheat planting, PRE herbicides are not possible to use and therefore weed control relies completely on POST herbicide applications. POST herbicides that provide acceptable weed control and tolerance to winter wheat could help advance this intercropping system in Georgia.

Appendix 1: Treatments for Italian ryegrass control in IMI-wheat at Plains, GA in 2007/2008[a]

| Treatment | Application timing | Rate |
|---|---|---|
| | | g ai $ha^{-1}$ |
| Non-treated | -- | -- |
| imazamox plus NIS | EMERG | 40+0.5%(v/v) |
| imazamox plus NIS | EMERG | 80+0.5%(v/v) |
| mesosulfuron plus NIS | EMERG | 20+0.25%(v/v) |
| mesosulfuron plus NIS | EMERG | 40+0.25%(v/v) |
| Diclofop | EMERG | 570 |
| Diclofop | EMERG | 1130 |
| imazamox plus NIS | POST | 40+0.5%(v/v) |
| imazamox plus NIS | POST | 80+0.5%(v/v) |
| mesosulfuron plus NIS | POST | 20+0.5%(v/v) |
| mesosulfuron plus NIS | POST | 40+0.5%(v/v) |
| Diclofop | POST | 570 |
| Diclofop | POST | 1130 |
| diclofop plus imazamox plus NIS | EMERG plus POST | 570+40+0.5%(v/v) |
| diclofop plus mesosulfuron plus NIS | EMERG plus POST | 570+20+0.25%(v/v) |
| diclofop plus diclofop | EMERG plus POST | 570+570 |

[a]Studies included cultivar GA-7T.

[b]All treatments except those only receiving diclofop included UAN at 4.73 L $ha^{-1}$.

[c]EMERGE treatments were made at crop emergence; POST treatments were made at Feekes stage 2.

Appendix 2. Treatments for efficacy study of ALS herbicides on conventional and IMI-wheat at Plains, GA in 2007/2008[a]

| Treatment | Rate |
|---|---|
| | g ai $ha^{-1}$ |
| Non-treated | -- |
| chlorsulfuron | 30 |
| chlorsulfuron | 60 |
| mesosulfuron plus UAN | 20+30%(v/v) |
| mesosulfuron plus UAN | 40+30%(v/v) |
| thifensulfuron plus NIS | 30+0.25%(v/v) |
| thifensulfuron plus NIS | 60+0.25% (v/v) |
| tribenuron plus NIS | 20+0.13%(v/v) |
| tribenuron plus NIS | 40+0.13%(v/v) |
| prosulfuron plus NIS | 30+0.13%(v/v) |
| prosulfuron plus NIS | 60+0.13%(v/v) |
| pyroxsulam plus COC | 190+1.25%(v/v) |

[a]Studies included cultivars GA-7T and AGS 2000.

[b]All treatments applied at label recommended timings.

[c]Chlorsulfuron treatments received MSO at 1.5 L $ha^{-1}$.

Appendix 3: Late season Italian ryegrass control in IMI-wheat at Plains, GA in 2007/2008[a]

| Treatment | Rate | Control |
|---|---|---|
| | g ai $ha^{-1}$ | % |
| imazamox plus NIS | 40+0.5%(v/v) | 45 |
| imazamox plus NIS | 80+0.5%(v/v) | 59 |
| mesosulfuron plus NIS | 20+0.25%(v/v) | 58 |
| mesosulfuron plus NIS | 40+0.25%(v/v) | 28 |
| diclofop | 570 | 84 |
| diclofop | 1130 | 94 |
| imazamox plus NIS | 40+0.5%(v/v) | 40 |
| imazamox plus NIS | 80+0.5%(v/v) | 67 |
| mesosulfuron plus NIS | 20+0.5%(v/v) | 48 |
| mesosulfuron plus NIS | 40+0.5%(v/v) | 60 |
| diclofop | 570 | 78 |
| diclofop | 1130 | 75 |
| diclofop plus imazamox plus NIS | 570+40+0.5%(v/v) | 77 |
| diclofop plus mesosulfuron plus NIS | 570+20+0.25%(v/v) | 90 |
| diclofop plus diclofop | 570+570 | 84 |

[a]Studies included the cultivar GA-7T.

[b]ANOVA indicated that there were no differences among treatment means.

Appendix 4. Percent crop stunting 7 days after application in winter wheat at Plains, GA in 2007/2008[a]

| Treatment | Rate | Crop Stunting | |
|---|---|---|---|
| | g ai $ha^{-1}$ | % | |
| chlorsulfuron | 30 | 0 | e |
| chlorsulfuron | 60 | 4 | cde |
| mesosulfuron plus UAN | 20+30%(v/v) | 9 | bc |
| mesosulfuron plus UAN | 40+30%(v/v) | 17 | a |
| thifensulfuron plus NIS | 30+0.25%(v/v) | 3 | de |
| thifensulfuron plus NIS | 60+0.25% (v/v) | 3 | de |
| tribenuron plus NIS | 20+0.13%(v/v) | 5 | bcde |
| tribenuron plus NIS | 40+0.13%(v/v) | 9 | bcde |
| prosulfuron plus NIS | 30+0.13%(v/v) | 0 | e |
| prosulfuron plus NIS | 60+0.13%(v/v) | 2 | de |
| pyroxsulam plus COC | 190+1.25%(v/v) | 7 | bcd |

[a]Studies included cultivars GA-7T and AGS 2000.

[b]Values followed by the same letter do not differ according to Fisher's protected LSD test ($P \leq 0.05$).

Appendix 5. AGS 2000 yield at Plains, GA for 2007/2008 season[a]

| Treatments | Rate | Yield |
|---|---|---|
| | g ai $ha^{-1}$ | kg $ha^{-1}$ |
| Non-treated | -- | 3316 |
| chlorsulfuron | 30 | 3118 |
| chlorsulfuron | 60 | 3282 |
| mesosulfuron plus UAN | 20+30%(v/v) | 2811 |
| mesosulfuron plus UAN | 40+30%(v/v) | 2923 |
| thifensulfuron plus NIS | 30+0.25%(v/v) | 3103 |
| thifensulfuron plus NIS | 60+0.25% (v/v) | 3175 |
| tribenuron plus NIS | 20+0.13%(v/v) | 3226 |
| tribenuron plus NIS | 40+0.13%(v/v) | 3279 |
| prosulfuron plus NIS | 30+0.13%(v/v) | 3247 |
| prosulfuron plus NIS | 60+0.13%(v/v) | 3257 |
| pyroxsulam plus COC | 190+1.25%(v/v) | 3179 |

[a]Study intiated in 2007.

[b]ANOVA indicated that there were no differences among treatment means.