EVALUATING COMMERCIAL CULTIVARS AND FARM-COLLECTED BIOTYPES OF ITALIAN RYEGRASS [*LOLIUM PERENNE* L. SSP. *MULTIFLORUM* (LAM.) HUSNOT] FOR POTENTIAL HERBICIDE RESISTANCE ISSUES IN GEORGIA

by

DANIELLE BURKE SIMMONS

(Under the Direction of Timothy L. Grey)

ABSTRACT

Italian ryegrass [*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot] is known for being the most troublesome weed in small grains in Georgia. This *Lolium* species is also a highly recommended cool-season forage that becomes problematic when total control is never achieved in warm-season bermudagrass or tall-fescue hayfields. Concerns about the lack of control in Italian ryegrass require Georgia populations be evaluated. Therefore, the response of Italian ryegrass populations to small grain herbicides was assessed. Greenhouse experiments from 2015 to 2017 indicate that post-emergence use herbicides lack control of some Georgia commercialized Italian ryegrass cultivars and farm-collected biotypes. A continuation of these experiments within the field setting are needed to determine if the responses can be replicated, and further analyses on other ryegrass populations should be conducted to determine if these trends are developing in experimental seed lots and currently available populations.

INDEX WORDS: Commercial cultivars, Control, Diclofop-methyl, Farm-collected biotypes, Flufenacet plus metribuzin, Glyphosate, Italian ryegrass, Pinoxaden, Pyroxasulfone, Pyroxsulam, Resistance

EVALUATING COMMERCIAL CULTIVARS AND FARM-COLLECTED BIOTYPES OF ITALIAN RYEGRASS [*LOLIUM PERENNE* L. SSP. *MULTIFLORUM* (LAM.) HUSNOT] FOR POTENTIAL HERBICIDE RESISTANCE ISSUES IN GEORGIA

by

DANIELLE BURKE SIMMONS

B.S., Abraham Baldwin Agricultural College, 2015

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

2017

EVALUATING COMMERCIAL CULTIVARS AND FARM-COLLECTED BIOTYPES OF ITALIAN RYEGRASS [*LOLIUM PERENNE* L. SSP. *MULTIFLORUM* (LAM.) HUSNOT] FOR POTENTIAL HERBICIDE RESISTANCE ISSUES IN GEORGIA

by

DANIELLE BURKE SIMMONS

Major Professor:	Timothy L. Grey
Committee:	William K. Vencill
	A. Stanley Culpepper

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
December 2017

DEDICATION

This Graduate Thesis is dedicated to the memory of Theodore M. Webster, who contributed greatly to my passion for Weed Science and to the field of Weed Science with respect to herbicide resistant species. I would also like to dedicate this to my parents and extended families for their love and support over the past two plus years.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Timothy Grey, Dr. William Vencill, and Dr. Stanley Culpepper for their assistance with creating protocols, performing statistical analysis, and editing drafts of this manuscript. For their technical support in developing experiments and harvesting and analyzing Italian ryegrass populations, I would like to acknowledge Sidney Cromer, Stuart Cook, Wellsley Martin, and Macey Connell.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

CHAPTER 1

INTRODUCTION

Herbicide resistance is the heritable ability of a plant to survive and reproduce after an application of herbicide that normally would have been fatal to all wild type individuals (Johnson et al. 2009; Vencill et al. 2012). Resistance within individuals can be naturally occurring or induced by mutagenesis, tissue culture variations, or genetic engineering (Jasieniuk et al. 1996; Christoffers 1999; Vencill et al. 2012). Increased herbicide use intensity, can be correlated to the increased number of herbicide-resistant weed species that currently exist (Vencill et al. 2012).

Within the past twenty years, researchers have suggested that the development of herbicide-resistant crops has resulted in the increased adoption of agronomic practices that depend less on mechanical manipulation of the soil [i.e. tillage] and more so on inputs like herbicide applications (Johnson et al. 2009; Vencill et al. 2012). Due to high selection pressure from herbicides with the same mode of action applied recently, certain weed species become more tolerant with increasing densities over time (Vencill et al. 2012). This shift in weed populations can eventually lead to herbicide resistant individuals (Johnson et al. 2009).

Herbicide-resistant weed species are a global issue exemplified in the case of Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot]. Italian ryegrass is a vigorous erect winter annual (SARE 2007; Bryson and DeFelice 2009) that has confirmed resistance in roadsides, small grain, lentil, soybean, corn, grape, grapefruit, and orchard cropping systems throughout the world (Heap 2017). The *Lolium* genus is known for rapidly evolving resistance to multiple herbicide mechanisms of action (Powles and Preston 2006). As of 2017, there are sixty

documented and confirmed instances of Italian ryegrass resistance across North America, South America, Europe, and Australia (Heap 2017). In Georgia, ryegrass resistance to diclofop-methyl, mesosulfuron-methyl, and sethoxydim has been confirmed in canola and wheat production systems (Heap 2017). However, little research has been done since the early 2000s to see if other active ingredients or mechanisms of action have developed potential herbicide resistance (Heap 2017). For this reason, research is needed to evaluate potential herbicide resistance in wild Italian ryegrass biotypes.

Although Italian ryegrass is a potentially devastating pest of small grains (Liebl and Worsham 1987; Appleby et al. 1976), in Georgia it is commonly grown as a winter forage crop for livestock production (Hancock 2013). Due to the high quality [18% CP (crude protein) and 70% TDN (total digestible nutrients)] at the vegetative stage (Hancock 2014), Italian ryegrass is favored over other cool-season annual grasses such as barley, oat, rye, and triticale (Hancock 2013; Hancock et al. 2015). Italian ryegrass is an ideal forage crop because it is easily digestible, very palatable to grazing animals, high in protein, and contains many minerals and vitamins (Lacefield et al. 2003). Livestock producers also favor Italian ryegrass in the southeast US because they can overseed it in bermudagrass [*Cynodon dactylon* L.] allowing them to maximize land usually dormant during the fall and winter months (Hancock 2014).

Since forage production and small grain production often occur on the same land over time, there are concerns that the planted ryegrass cultivars for forage may consist of resistance mechanisms to various herbicides including those used for control in small grains. If out-crossing of a resistant trait during pollination of the forage or seed production of the crop were to occur, small grain production may be eliminated. Thus, it is critical to understand if commercialized ryegrass cultivars contain resistance to herbicides used for its control in small grains.

CHAPTER 2

LITERATURE REVIEW

Species History

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], also known as 'Westerwolds' or annual ryegrass, is a monocot species that is a member of the Poaceae family (Lacefield et al. 2003; OGTR 2008; Heap 2017). Belonging to the subfamily Pooideae, the *Lolium* genera is one of thirty-nine within the tribe *Poeae* (Wheeler et al. 2000; Wheeler et al. 2002). The *Lolium* genera is sometimes taxonomically grouped together with *Festuca* due to the spicate inflorescence of Italian ryegrass mutating into a paniculate form thus erasing any morphological difference between the two genera (Jauhar 1993).

This weed species has its origins in temperate regions of southern Europe where the first reported cultivation of planted material occurred in northern Italy in the 13th or 14th century [year unknown] (Beddows 1953; Suvorova 1960). Following this initial report, the presence and cultivation of Italian ryegrass was soon recorded in France (Lacefield et al. 2003), Switzerland (Stebler and Volkart 1913), and England (Terrell 1968) between 1818 to 1831 (Lacefield et al. 2003). It was imported and introduced as a forage crop during the early Colonial period into North America (Lacefield et al. 2003). Since this introduction, Italian ryegrass has become an important cool-season forage grass in the southern region of the United States (Lacefield et al. 2003) due to its preferential adaption to moist, cool environments (Lacefield et al. 2003; Romani et al. 2002).

Weedy Characteristics

Italian ryegrass is considered a facultative, winter annual grass species that infests both winter and spring planted crops (Rauch et al. 2010). It is distributed throughout all regions of the United States due to its adaptability to a wide range of soil types and temperatures (USDA NRCS 2017). However, this cool-season species thrives greatly in temperate, mild climates with rich soils [pH of 6.0 to 7.0] and cannot endure large temperature fluctuations such as severely wet, cold or abnormally hot, dry weather conditions (USDA NRCS 2017; Lacefield et al. 2003). For example, Italian ryegrass in Australia is grown under non-irrigated and irrigated conditions within temperate regions (Bolland et al. 2001). Under normal growing conditions, Italian ryegrass seedlings will mature in the fall, use a vegetative state to overwinter, and resume active development in the spring (McCullough 2015).

Italian ryegrass' aggressive growth pattern is characterized by several distinguishing features. This plant has an extensive fibrous root structure that allows for quick establishment over many other grass species (Bryson and DeFelice 2009; SARE 2007; Ball et al. 1995). Due to its root structure, Italian ryegrass can reach approximately 1.3 m in height (Bryson and DeFelice 2009; USDA NRCS 2017), which allows it to protrude above wheat canopies and interfere with light uptake by the crop (Ball et al. 1995). With respect to above-ground development, a single mature plant can produce multiple tillers extending from 6.0 to 36.0 cm long and 4.0 to 10.0 cm wide with long, clasping auricles. Multiple seed heads with awned seeds are also produced (Bryson and DeFelice 2009).

As an obligate out-crosser (Lacefield et al. 2003; Inoue et al. 2014) that reproduces by abundant seed production [102,000 seed kg^{-1}] (Lacefield et al. 2003), Italian ryegrass is particularly prone to evolving resistance to herbicides (Heap 2017; Terrell 1968). Wild-type (Powles et al. 1998) and commercial Italian ryegrass populations can contain a multitude of gene variations, which allow these individuals to possess inter-population variability (Inoue et al. 2014). When *Lolium* biotypes cross-pollinate, offspring can form genetically diverse individuals that potentially contain inherent resistance mechanisms within their genome (Preston and Powles 2002; Powles et al. 1998).

Its abundant seed production and growth pattern contribute to Italian ryegrass being the most troublesome and the fourth most common weed in small grains in Georgia, Alabama, and Arkansas (Webster 2012), where approximately 14,000 ha of rye and 392,000 ha of wheat were harvested in 2012 (USDA 2012). Italian ryegrass infestations of 29 to 118 plants m^{-2} have been documented to reduce overall wheat yields from 7 to 50% (Appleby et al. 1976). Since competition from Italian ryegrass can reduce wheat yields by 4.2% for every 10 plants m^{-2} (Liebl and Worsham 1987), the need for adequate Italian ryegrass control is vital for maximum yield production in small grain crops (Hively and Cox 2001; Reddy 2001; Cralle et al. 2003).

Documented Cases of Resistance

Herbicides are the most efficient and economical means of control for Italian ryegrass in small grains (Appleby et al. 1976; Justice et al. 1994; Kuk et al. 2008). However, with increased herbicide and herbicide-resistance crop usage (Owen and Zelaya 2005; Young 2006) and a range of resistance mechanisms already identified in Italian ryegrass (Betts et al. 1992), resistance

probability increases. With respect to small grain cropping systems in Georgia, ACCase-resistant Italian ryegrass was first documented in 1995 in canola and wheat, resistance to ACCase- and ALS-inhibiting herbicides was reported in wheat in 2009, and resistance to yet another ACCase herbicide was documented in 2010 (Heap 2017).

Ryegrass is resistant to 7 herbicide mechanisms of action (Heap 2017) with resistance documented in post-emergence use pattern herbicides (Grey and Newsom 2017), except for one report in Australia (Busi et al. 2014). Resistance within *Lolium rigidum*, a similar species of annual ryegrass, was confirmed to the pre-emergence use herbicide pyroxasulfone (Busi et al. 2014). Pyroxasulfone is a relatively new pesticide [2000s] that is considered an excellent selective herbicide in small grain crops for the control of both broadleaf and grass weed species (Busi et al. 2014; Tanetani et al. 2009). The herbicide inhibits very long chain fatty acid elongases by blocking lipid biosynthesis processes, which in turn inhibits shoot elongation in germinated weed seedlings (Busi et al. 2014; Porpiglia et al. 2006; Kobayashi et al. 2007; Böger et al. 2000). Although this pesticide has shown great potential, an experiment conducted on *Lolium rigidum* prior to pyroxasulfone's commercialization found that resistance had evolved within three generations after re-current low doses (Busi et al. 2014). A previous study conducted by Busi et al. (2014) confirmed that the resistance mechanism in *Lolium rigidum* was a semi-dominant allele that segregated from a major locus within the genome.

ACCase-resistance. The first documented herbicide resistance of Italian ryegrass was to diclofop-methyl, an ACCase-inhibiting herbicide, in Oregon in 1987 (Heap 2017; Stanger and Appleby 1989). ACCase, or acetyl Coenzyme-A carboxylase, is the key enzyme in the biosynthesis of fatty acids in plants, and ACCase-inhibiting herbicides block the production of phospholipids used in building new membranes required for cell growth (Shimabukuro 1990; Vencill 2002).

Evidence suggests that the appearance of herbicide resistance within a weed population is due to the pressure exerted by repeated herbicide applications of the same mechanism of action (Hirschberg and McIntosh 1983; Roux and Reboud 2007). Repeated use of ACCase-inhibiting herbicides belonging to the chemical subfamilies cyclohexanedione (CHD) and aryloxyphenoxy propionate (AOPP), such as diclofop-methyl, has resulted in resistant biotypes (Eleni et al. 2000; Kuk et al. 2008). Italian ryegrass resistant to ACCase-inhibiting herbicides like diclofop-methyl has been confirmed in Oregon, Arkansas, Louisiana, Tennessee, Georgia, Virginia, Maryland, Kentucky, North and South Carolina, and Idaho (Heap 2017; Kuk and Burgos 2007). Diclofop-methyl resistance is due to a mutation of aspartate-to-glycine or isoleucine-to-asparagine substitutions at positions 2078 and 2041, respectively, within the acetyl CoA carboxylase genome (Kuk et al. 2008).

Resistance to other ACCase chemistries such as clodinafop-propargyl, quizalofop-p-ethyl, clethodim, and sethoxydim has been confirmed in Idaho Italian ryegrass populations (Heap 2017; Rauch et al. 2010). Fenoxaprop-p-ethyl (Heap 2017) and pinoxaden resistance (Kuk et al. 2008) have been confirmed in Arkansas and Missouri, respectively.

ALS-resistance. Within ten years of documenting ACCase resistance (Heap 2017), ALS resistant Italian ryegrass was confirmed throughout the US (Kuk et al. 2008). ALS, or acetolactate synthase, is the first enzyme in biosynthesis of branched chain amino acids such as valine, leucine, and isoleucine and the inhibition of these amino acids can disrupt protein synthesis and inhibit plant growth (Yu et al. 2003). Italian ryegrass resistance has been confirmed for various ALS herbicides like triasulfuron in Idaho (Heap 2017) and sulfometuron-methyl in Mississippi (Taylor and Coats 1996).

Resistance to other ALS herbicides such as mesosulfuron-methyl in Georgia, Delaware, and Missouri, and to mesosulfuron-methyl and pyroxsulam in Kentucky have been confirmed (Heap 2017; Rauch et al. 2010); sulfometuron-methyl, mesosulfuron-methyl, chlorsulfuron, and imazamox in Arkansas indicating that these populations have cross-resistance to other ALS chemistries (Heap 2017; Kuk and Burgos 2007); within the Carolina region, North Carolina Italian ryegrass populations exhibited ALS resistance to mesosulfuron-methyl, imazamox, and pyroxsulam (Chandi et al. 2011; Heap 2017); and in South Carolina resistance to mesosulfuron-methyl and pyroxsulam (Heap 2017). ALS resistance in Italian ryegrass was discovered to be a type of target site resistance due to a mutation in the acetolactate gene at proline 197 (Yu et al. 2003).

EPSP-resistance. Due to the effectiveness and extensive long-term use of glyphosate, inherent resistance mechanisms within certain weed species have rapidly expressed themselves (Perez-Jones et al. 2005). Glyphosate is a nonselective, broad-spectrum herbicide that inhibits the enzyme 5-enolpyruvlshikimate-3-phosphate synthase [EPSPS], which results in the inhibition of aromatic amino acids and protein biosynthesis (Nandula et al. 2008; Franz et al. 1997). Within the US, glyphosate resistant Italian ryegrass has been confirmed in Oregon, Louisiana,

Mississippi, Arkansas, Tennessee, North Carolina, and California (Bond et al. 2014; Dickson et al. 2011; Heap 2017; Perez-Jones et al. 2005). It was determined that resistance to glyphosate occurred as a result of metabolic mechanisms influencing the amount of shikimic acid produced and the translocation and absorption of the glyphosate in the plant (Nandula et al. 2008; Perez-Jones et al. 2005).

One notable case of glyphosate resistance abroad is that of confirmed *Lolium* resistance in Spain, in which 97% of the resistant biotype survived the field use rate (720 g ae ha^{-1}). Within this study, it was noted that the accumulation and translocation of glyphosate was much lower [2 to 5 times] in the resistant biotype than the susceptible biotype (Fernández-Moreno et al. 2017).

Cross-resistance. As previously discussed, some Italian ryegrass populations can select for resistance to two or more herbicides with a single mechanism of action which gives them the ability to survive herbicide applications from different chemical families (Kuk et al. 2008; Vencill et al. 2012). Cross-resistance among the ACCase herbicides is a common issue in Italian ryegrass populations (Michitte et al. 2003; Rauch et al. 2010; Heap 2017), but is more often observed after diclofop-methyl resistance has been selected for (Tardif et al. 1993). However, cross-resistance is not limited to ACCase chemistries as it has been documented to occur with ALS chemistries in some Italian ryegrass populations (Kuk and Burgos 2007; Rauch et al. 2010; Heap 2017).

Multiple-resistance. In some incidences, Italian ryegrass biotypes resistant to ALS-inhibiting herbicides can develop resistances to herbicides that affect different target sites such as ACCase and vice versa (Holtum and Powles 1991; Eleni et al. 2000; Kuk et al. 2008; Salas et al. 2013). These populations are said to develop multiple resistance, which occurs when a weed species develops multiple resistance mechanisms (Powles and Prestion 1995) to two or more

herbicides with multiple mechanisms of action (Vencill et al. 2012). Multiple resistance has occurred with other chemistries such as glyphosate wherein resistance has been reported in many of the same populations that are either ACCase-resistant, ALS-resistant, or resistant to both mechanisms of action (Heap 2017).

Objectives

Concerns have been expressed to researchers that ryegrass cultivars sown for forage may consist of resistance to various herbicides used for control in small grain production and may eliminate production if resistance traits are spread during pollination. Therefore, it is vital that research be conducted to address this subject. The first objective of this research is to determine the response of commercialized Italian ryegrass cultivars to small grain herbicides.

Few herbicide surveys have been conducted to determine the extent of potential Italian ryegrass herbicide resistance within the state of Georgia, which accounts for limited information being available. In addition, a complete screening of the different herbicide mechanisms of action with confirmed resistance has not been conducted. Therefore, the second objective of this research is to determine the response of Georgia Italian ryegrass populations to small grain herbicides.

CHAPTER 3

HERBICIDE EFFECTS ON ITALIAN RYEGRASS [*LOLIUM PERENNE* L. SSP. *MULTIFLORUM* (LAM.) HUSNOT] CONTROL: 2015 and 2016 COMMERCIAL CULTIVAR TRIALS[1]

[1] Simmons, D.B., T.L. Grey, W.K. Vencill, and A.S. Culpepper. To be submitted to *Weed Technology*.

Abstract

Greenhouse experiments evaluated the response of commercially grown forage cultivars of Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] to small grain herbicides. Experiments were conducted from fall 2015 through spring 2017 in Athens, and Tifton, Georgia. In 2015 twenty-six cultivars were evaluated while fifty-nine cultivars were included in 2016. Pyroxasulfone or flufenacet plus metribuzin were applied PRE to Italian ryegrass cultivars at 59 and 477 g ai ha^{-1}, respectively. Diclofop-methyl (841 g ai ha^{-1}), glyphosate (875 g ae ha^{-1}), pinoxaden (61 g ai ha^{-1}), or pyroxsulam (19 g ai ha^{-1}) were applied to ryegrass cultivars at Feekes' stage 1.0 (1 to 2 leaf). A non-treated control for each cultivar and herbicide use pattern was included for comparison. For both experimental years, all commercial cultivars were controlled by the applications of pyroxasulfone or flufenacet plus metribuzin. Initial biomass displayed that all cultivars were controlled by the POST applications. Data analysis for the 2015 experiments demonstrated that a lack of control in Italian ryegrass cultivars has occurred for the diclofop-methyl, glyphosate, or pyroxsulam applications amongst certain regrowth data measurements. A similar response in control has occurred across the regrowth data for the diclofop-methyl, glyphosate, pinoxaden, or pyroxsulam applications in the 2016 experiments. A continuation of these experiments within the field setting would better determine the response of commercially available cultivars to small grain herbicides. Further analyses should be conducted to determine if lack of control is developing in experimental Italian ryegrass seed lots.

Nomenclature: Diclofop-methyl; flufenacet plus metribuzin; glyphosate; pinoxaden; pyroxasulfone; pyroxsulam; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot

Key Words: Commercialized cultivars, greenhouse experiments, ryegrass control, small grain herbicides

Introduction

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], also known as annual ryegrass, is a vigorous erect winter annual grass species found in temperate regions throughout the world (SARE 2007; McCullough 2015; Bryson and DeFelice 2009). Within small grain cropping systems and spring seeded forage fields, Italian ryegrass is considered an aggressive weed species that can cause significant yield loss and stand reduction (McCullough 2015). According to McCullough (2015), Italian ryegrass can reduce bermudagrass [*Cynodon dactylon* L.] or tall fescue [*Lolium arundinaceus* (Schreb.) Darbysh] hayfield yields by as much as 50% or more in some areas. Despite the weedy aspect of this species, Italian ryegrass is a highly recommended cool-season forage crop for livestock production within Georgia (Hancock 2013).

Originating in Europe, the migration of Italian ryegrass to the US occurred in Colonial times for use as a forage crop for livestock production (Lacefield et al. 2003). Today, there are more than fifty commercially available cultivars that range from early, late, and season-long grazing varieties (Hancock 2013; Hancock 2014). Under normal growing conditions, Italian ryegrass seedlings will mature in the fall, use a vegetative state to overwinter, and resume active development in the spring (McCullough 2015).

Researchers have noted that the high quality (Lacefield et al. 2003; Hancock 2013) and prolific seed production of Italian ryegrass make it an ideal forage crop (Hancock 2014; McCullough 2015; Terrell 1968). With respect to its below-ground development, Italian ryegrass has an extensive fibrous root structure (Bryson and DeFelice 2009; SARE 2007) which increases uptake of water and nutrients (Stone et al. 1998) allowing for it to flourish even under non-irrigated conditions (Bolland et al. 2001). This well-adapted species is easily established and

tolerates poor drainage conditions unlike other forage crops such as clovers [*Trifolium repens, incarnatum, vesiculosum* L.] and bermudagrass [*Cynodon dactylon* L.] (Hancock 2013). As many as three high yield silage cuttings can be obtained from a single season of annual ryegrass production (Wilkins and Humphreys 2003). Italian ryegrass allows for an extended grazing period as compared to other forage crops, which means livestock can graze until May in South Georgia and as late as early June in North Georgia (Hancock 2013).

The earliest US commercial cultivars released were 'Gulf' by the USDA-ARS in Texas in 1958, 'Florida Rust Resistant' by the Florida AES in 1965, and 'Marshall' by the Mississippi AES in 1980 (Blount and Prine 2016). Today's varieties recommended for Georgia forage production such as 'Attain', 'Big Boss', 'Diamond T', 'Fria', 'Nelson', etc. (Hancock 2014) are from newer breeding germplasms [yr. 2000 +] (Blount and Prine 2016). The total number of germplasms, or genetic resource collections, for Italian ryegrass breeding is 2,660 which can be attributed to its allogamous reproductive pattern. The purpose of these breeding procedures is to improve the palatability, digestibility, nutritional value, disease resistance, yield potential, and winter hardiness of Italian ryegrass (Inoue et al. 2014).

Commercially available cultivars are classified by their ploidy level as either diploid [2x] or autotetraploid [4x] (Lacefield et al. 2003; Anonymous 2015; Inoue et al. 2014). The original commercial cultivars were diploid cultivars that are recommended for sowing and grazing as a stand-alone forage crop. These varietal types are described as having larger tiller density than tetraploid types which allows for lower seeding rates and the ability to overcome potential weed suppression (Anonymous 2015). Tetraploid commercial cultivars are newer varieties that are recommended for fodder conservation practices and legume forage mixtures. Tetraploid types

have larger seeds, larger leaves, and higher fresh yields than diploid types. Even though this varietal type establishes faster than diploid varieties, it requires a 25 to 40% higher seeding rate (Anonymous 2015).

Ryegrass cultivars are further classified by maturation levels such as early-mid, mid-late, and season-long [late maturing] varieties (Hancock 2013; Anonymous 2015). Early-mid season varieties are short season varieties recommended for quick feedings in the winter and in early spring fodder conservation practices. These varieties are suited for low rainfall areas or areas where high rainfall and summer forage crops are sprayed and sowed (Anonymous 2015). Mid-late season varieties are recommended for medium to high rainfall areas where a second cut of fodder or late grazing can occur. Late maturing, or season-long, varieties are recommended for long growing season areas that produce feed for 1 to 2 years after sowing. This varietal type is good for heavy rainfall areas and can produce higher quality feed later into the grazing season (Anonymous 2015).

However during spring emergence of warm-season forages, Italian ryegrass is the most challenging winter annual weed species (McCullough 2015). To control escaped Italian ryegrass, a combination of pre-plant incorporated [PPI], pre-emergence [PRE], and post-emergence [POST] herbicides are recommended in non-ryegrass forage fields (McCullough 2017). These recommended herbicides have several different mechanisms of action including acetyl Coenzyme-A carboxylase [ACCase], acetolactate synthase [ALS], photosystem I [PSI], 5-enolpyruvlshikimate-3-phosphate synthase [EPSPS], growth regulators, and mitotic inhibitors (McCullough 2015). The consecutive, repeated application of herbicides with these mechanisms of action has created instances where Italian ryegrass populations are suspected of selecting for herbicide resistance (Busi et al. 2014).

Previous research has shown ryegrass populations in the US Pacific Northwest, where a majority of Italian ryegrass seed is grown and harvested for retail, have confirmed resistance to multiple herbicide mechanisms of action that include ACCase (graminicides like diclofop-methyl), ALS (sulfonylureas and imidazolinones), EPSP synthase (glyphosate), very long chain fatty acid [VLCFA] (pyroxasulfone), Photosystem II [PSII] (triazines), and glutamine synthetase [GS] (glufosinate) (Mallory-Smith et al. 2015; Heap 2017). Overall, there is currently limited information available on herbicide screenings of commercial cultivars. The purpose of this research is to determine the response of commercially available Italian ryegrass cultivars grown in Georgia to small grain herbicides.

Materials and Methods

Seed Collection. Twenty-six and fifty-nine commercially available Italian ryegrass cultivars, commonly used in forage production, were obtained from the University of Georgia Variety Testing in 2015 and 2016, respectively. No specific information about cultivars was made available. Subsamples of seed from each cultivar accession were stored at 6 C until tested. To ensure adequate germination, seed were allowed to acclimate to 20 C over a 7 d period prior to planting.

Greenhouse Experiment (2015). An experiment was conducted twice from winter 2015 to spring 2016 at the College Experiment Station, Athens, Georgia, and Coastal Plains Experiment Station, Tifton, Georgia. Ryegrass seeds were sown into 237 mL cups at both locations. In Athens, cups were filled with a 1:3 ratio of steam sterilized Cecil sandy loam

[72% sand, 12% silt, 16% clay; fine, kaolinitic, thermic Typic Kanhapludults] with pH of 5.5 and 2.1% organic matter to pure sand. Adding sand allowed for the overall soil structure to more closely resemble the structure of the soil used at the Tifton location which included a steam-sterilized Tifton loamy sand [90% sand, 6% silt, 4% clay; fine-loamy, kaolinitic, thermic Plinthic Kandiudults] with pH 5.6 and 1.0% organic matter.

For both experimental locations, a teaspoon was used to sprinkle 20 to 30 seed of each cultivar over the soil surface within each cup. A quarter inch of the respected soil was placed on top of the sown seeds. Each teaspoon of seed had an average weight of 0.13 g. Each 237 mL cup had five holes poked randomly into the bottom with a 16 penny nail for drainage purposes. For the duration of these experiments, the cups were placed into greenhouses prior to and after completion of herbicide applications.

All plants within the Athens greenhouse were fertilized weekly with a common 5-10-15 granular fertilizer and watered twice a day for 2 minutes until field capacity. In Tifton, plants were fertilized weekly with 24-8-16 Miracle-Gro liquid fertilizer[1] and watered once a day for 5 minutes until field capacity. Supplemental light of 500 μmol m^{-2} s^{-1} light intensity was provided when necessary, and heaters kept the temperature between 25 to 29 C. These maintenance steps were taken to ensure that any symptomology, displayed by the Italian ryegrass seedlings, was due to the efficacy of the herbicide and not environmental stressors [i.e. lack of water, nutrients, etc.].

Pyroxasulfone or flufenacet plus metribuzin were applied PRE within 0 to 1 d of planting at 59 and 477 g ai ha^{-1}, respectively. Diclofop-methyl (841 g ai ha^{-1}), glyphosate (875 g ae ha^{-1}), or pyroxsulam (19 g ai ha^{-1}) were applied to emerged ryegrass at Feekes' stage 1.0 (Wise et al. 2011) or 7 d after planting. Recommended adjuvants COC (1% v/v) and UAN (2% v/v) were

included in the pyroxsulam application. Herbicide rates corresponded to the recommendations for labeled uses in wheat [*Triticum aestivum* L.] cropping systems (Culpepper 2017). All herbicide application rates were made with a compressed air spray chamber at both locations. In Athens, the compressed air chamber delivered 200 L ha^{-1} volume of water at 241 kPa at 5 kph [nozzle unknown]. Meanwhile at the Tifton location, the chamber delivered 187 L ha^{-1} volume of water at 138 kPa at 3 kph using a XR Teejet 11002 VS nozzle. A non-treated control for each cultivar and each herbicide application scenario [PRE vs. POST] was included in the experiment.

For locations receiving the PRE treatments, ryegrass seedlings that emerged after application were harvested 21 DAT by clipping the shoots at soil level and taking a fresh weight biomass measurement. All collected samples were placed into a drier at 32 C for 168 hours [Athens] and 95 C for 72 hours [Tifton], after which dry weight biomass measurements were recorded.

For the POST herbicide treatments, all above-ground biomass was harvested 21 DAT by clipping all necrotic and non-necrotic shoots at soil level. A fresh weight biomass measurement was taken for each sample. All collected samples were placed into a drier at 32 C for 168 hours [Athens] and 95 C for 72 hours [Tifton]. Dry weight biomass measurements were then recorded for each of the samples.

Following the initial 21 DAT harvest at the Tifton location, Italian ryegrass seedlings of POST treatments were allowed to regrow for 14 d. After this regrowth period, stand counts or counts of emerged shoots in each cup were recorded. Emerged shoots were harvested and biomass data was collected using similar methodology from the initial harvest at 21 DAT. Roots from each cup were also washed free of soil and drained of excess water. Each of the root

samples had a fresh weight biomass measurement recorded before placing them into a drier at 95 C for 72 hours, after which dry weight biomass measurements were documented. Due to high variability between cultivars, this additional harvest was not performed at the Athens location.

Greenhouse Experiment (2016). An experiment was conducted twice from the winter 2016 to spring 2017 at the Coastal Plains Experiment Station, Tifton, Georgia. For PRE herbicide treatments, ryegrass was planted into 18 cell plastic flats [51cm x 26cm x 6cm] following the same procedures as noted with the 2015 PRE treatments. The experimental container was altered due to space constraints in the greenhouse, wherein each flat contained 18 of the tested cultivars allowing for less space to be occupied. To ensure similar amounts of seeds were planted, a teaspoon was used to sprinkle 30 to 40 seed of each cultivar over the soil surface within each of the cells. A quarter inch of the respected soil was placed on top of the sown seeds. Each teaspoon of seed had an average weight of 0.19 g.

For the POST herbicide treatments, seeds were sown using the same methodology as the PRE treatments except a 1:1 ratio of steam-sterilized Tifton loamy sand to potting soil [45 to 55% composted bark fines, reed sedge, peat, other composited wood products and perlite][2] was used. Adding potting soil allowed for less soil leaching and increased soil retention in the flats, which ensured that all POST herbicide chemistry applied was readily available for uptake. Due to the influence of organic matter on the chemistries of PRE herbicides, it was not recommended that potting soil be added as it reduces the efficacy potential of the herbicides. Greenhouse procedures including temperature, watering, and fertilization followed methods noted for the 2015 experiments.

Pyroxasulfone or flufenacet plus metribuzin were applied PRE within 0-1 d of planting at 59 and 477 g ai ha^{-1}, respectively. Diclofop-methyl (841 g ai ha^{-1}), glyphosate (875 g ae ha^{-1}), pinoxaden (61 g ai ha^{-1}), or pyroxsulam (19 g ai ha^{-1}) were applied to emerged ryegrass at Feekes' stage 1.0 (Wise et al. 2011) or 7 d after planting. Recommended adjuvants COC (1% v/v) and UAN (2% v/v) were included in the pyroxsulam application. Herbicide rates corresponded to the recommendations for labeled uses in wheat [*Triticum aestivum* L.] cropping systems (Culpepper 2017). All herbicide application rates were made with a compressed air spray chamber that delivered 187 L ha^{-1} volume of water at 138 kPa at 3 kph using a XR Teejet 11002 VS nozzle. A non-treated control for each cultivar and each herbicide application scenario [PRE vs. POST] was included in the experiment.

For locations receiving the PRE treatments, ryegrass seedlings that emerged after application were harvested 21 DAT by clipping the shoots at soil level and taking a fresh weight biomass measurement. All collected samples were placed into a drier at 95 C for 72 hours, after which dry weight biomass measurements were recorded.

For the POST herbicide treatments, all above-ground biomass was harvested 21 DAT by clipping all necrotic and non-necrotic shoots at soil level. A fresh weight biomass measurement was taken for each sample. All collected samples were placed into a drier at 95 C for 72 hours. Dry weight biomass measurements were then recorded for each of the samples.

Following the initial 21 DAT harvest, Italian ryegrass seedlings of POST treatments were allowed to regrow for 14 d. After this regrowth period, stand counts or counts of emerged shoots in each individual cell were recorded. Emerged shoots were harvested and biomass data was collected using similar methodology from the initial harvest at 21 DAT. Roots from each

cup were also washed free of soil and drained of excess water. Each of the root samples had a fresh weight biomass measurement recorded before placing them into a drier at 95 C for 72 hours, after which dry weight biomass measurements were documented.

Experimental Design

There were two site locations for the 2015 commercial cultivars, Athens and Tifton, Georgia, and one location, Tifton, Georgia, for the 2016 commercial cultivars. Experiments were arranged as a split-plot design with four replications of 6 herbicide treatments for the 2015 commercial cultivars. In 2016, the increase in cultivars planted allowed only three replications of each of the 7 herbicide treatments arranged as a split-plot design. Experiments were repeated twice at each location. Within the split-plot arrangement, the herbicide treatments represent the whole plot and the cultivars represent the subplot.

Analyses of all recorded data was combined across experiments, replications, and locations [2015] and subjected to a mixed-model analysis with an alpha of $P \leq 0.05$ in SAS 9.4 ®. Initial, fresh shoot biomass obtained from all PRE treatments and the respective non-treated controls was analyzed via a PROC GLM procedure. Additionally, initial, fresh shoot biomass and regrowth shoot emergence, fresh shoot biomass, and fresh root biomass data recorded for all POST treatments and the respective non-treated controls were analyzed via a PROC GLM procedure. For the PROC GLM procedure, the main effects were the herbicide treatments and ryegrass cultivars, the two-way interaction of interest was the herbicide treatment by cultivar, and the error term was determined to be the two-way interaction of herbicide treatment by experiment. This analysis procedure was chosen due to the large number of cultivars tested per experiment.

Results and Discussion

Due to differences in experimental design already established, each experimental year is discussed individually. Upon analysis, data indicated that the two-way interaction of treatment by cultivar varied amongst the experimental years in significance. There were no significant interactions for treatment by cultivar, except for the initial, fresh shoot biomass of the POST applied herbicides in the 2015 analysis. However for the 2016 analysis, there were more significant treatment by cultivar interactions than not. For these reasons, the main effects of treatment and cultivar and the two-way interaction of treatment by cultivar P-values are documented. Dry biomass data will not be presented for these experiments because the trends in data correspond to those displayed by the fresh biomass data.

2015. For the PRE treatments flufenacet plus metribuzin or pyroxasulfone and their respective non-treated controls, the initial, fresh shoot biomass analysis indicated that there were no differences for the two-way treatment by cultivar interaction (P = 0.8943) (Table 3.1). However, the treatment variable response was significant at P = 0.0002 (Table 3.1) with an LSD of 556 mg exp unit^{-1} (Table 3.2). The non-treated control had the largest mean at 1623 mg exp unit^{-1}, while flufenacet plus metribuzin had the smallest mean shoot biomass at 75 mg exp unit^{-1} (Table 3.2). These conclusions differ from results of previous experiments completed by Busi et al. (2014) that confirmed lack of control of the Australian ryegrass cultivar SLR31 to pyroxasulfone, but correspond with previous research that applications of flufenacet plus metribuzin at PRE or early POST provide 80% or greater control of Italian ryegrass (Grey and Bridges 2003; Ritter and Menbere 2002).

Analysis of the cultivar response (P = 0.6822) to the PRE treatments displayed that there were no differences between the twenty-six cultivars when combined across treatments (Table 3.1). Yet with an LSD of 377 mg exp unit^{-1}, there were differences in shoot biomass between the commercialized Italian ryegrass cultivars. Mean shoot biomass across cultivars ranged from 657 to 1301 mg exp unit^{-1} with the 'Marshall' cultivar and the 'Credence (IS-LWT 14)' cultivar having the smallest and largest biomasses, respectively (Table 3.3). Varying shoot biomass between cultivars concurs with previous performance tests on commercialized ryegrass cultivars conducted by UGA Statewide Variety Testing (Anonymous 2017).

Across the POST treatments of diclofop-methyl, glyphosate, or pyroxsulam and their respective non-treated controls, the analysis of the initial, fresh shoot biomass displayed that there were significant differences in treatment variable response with P = 0.0110 (Table 3.4) and an LSD of 0.37 g exp unit^{-1} (Table 3.5). The two-way treatment by cultivar interaction with P = 0.0028 was also significant (Table 3.4). For the treatment variable, the diclofop-methyl application had the smallest mean shoot biomass at 0.21 g exp unit^{-1}, whereas the non-treated control's mean was the largest at 1.15 g exp unit^{-1} (Table 3.5). Previous research on Italian ryegrass accessions indicated similar control of ryegrass with glyphosate applications (Kuk and Burgos 2007), but differ from previous experiments conducted by Chandi et al. (2011) and Salas et al. (2010) on variable and decreased control of Italian ryegrass with diclofop-methyl and pyroxsulam, respectively.

The analysis concluded that there was a significant cultivar response ($P < 0.0001$) when cultivars were combined across treatments for the shoot biomass (Table 3.4). With an LSD of 0.13 g exp unit^{-1}, the commercial cultivars had a mean range of 0.38 to 0.73 g exp unit^{-1}. Similar to the results of the PRE experiments, the 'Credence (IS-LWT 14)' cultivar had the largest biomass and the 'Marshall' cultivar had the smallest for the POST treatments (Table 3.6).

For the regrowth shoot emergence data of the POST treatments and their respective non-treated controls, there were no differences for the two-way treatment by cultivar interaction ($P = 0.4230$) (Table 3.4). Yet, there were significant differences for the treatment response ($P = 0.0262$) (Table 3.4) which had an LSD of 8 shoots (Table 3.5). The largest and smallest mean shoot emergence counts were 19 plants for the non-treated control and 3 plants for the diclofop-methyl treatments, respectively, which resembled the results of the initial, fresh shoot biomass analysis (Table 3.5). On closer analysis of the treatments, the difference in shoot emergence means for glyphosate and the non-treated control was less than the LSD. This concurs with previous experiments indicating lack of control of Italian ryegrass populations by applications of glyphosate (Perez-Jones et al. 2005; Jasieniuk et al. 2008). Results differ however from research conducted by Salas et al. (2010) that documented lack of control by pyroxsulam and research by Chandi et al. (2011) that stated ryegrass control was variable when diclofop-methyl was applied.

There were no differences for the cultivar variable ($P = 0.0755$) for shoot emergence counts of the POST treatments (Table 3.4). Although the analysis displayed no differences, the LSD of 3 plants exhibited differences amongst the cultivars. The 'Jumbo' cultivar had the smallest mean shoot emergence count at 7 plants, while the 'Flying A', 'GO-15-LN2', 'Lonestar', and 'Nelson Tetraploid' cultivars all had the largest mean of 12 plants (Table 3.6).

Analysis on the regrowth shoot biomass for the POST treatments displayed that there were no differences in treatment response (P = 0.7075) or the two-way treatment by cultivar interaction with P = 0.4198 (Table 3.4). Even though the treatment LSD was determined to be 100 mg plant^{-1}, the diclofop-methyl and pyroxsulam applications had mean shoot biomass of 55 and 37 mg plant^{-1}, respectively, as compared to the non-treated control mean of 27 mg plant^{-1} (Table 3.5). This data suggest that the diclofop-methyl (Chandi et al. 2011) and pyroxsulam (Kuk and Burgos 2007; Salas et al. 2010) applications lacked control of the Italian ryegrass cultivars which would concur with previously conducted experiments using these herbicides. However, the regrowth shoot biomass data agrees with experiments conducted by Kuk and Burgos (2007) in which glyphosate applications adequately controlled Italian ryegrass populations.

With P = 0.5861, there were determined to be no differences in cultivar response for the regrowth shoot biomass, but there were differences in regrowth shoot biomass between the cultivars (Table 3.4). Regrowth shoot biomass means were the largest at 54 mg plant^{-1} for the 'Prine' cultivar and the smallest for the 'Winterhawk' cultivar at 22 mg plant^{-1}. With respect to the regrowth shoot biomass means, the LSD was 25 mg plant^{-1}(Table 3.6).

The regrowth root biomass analysis, of the POST treatments and their respective non-treated controls, resulted in no differences for the treatment variable response (P = 0.1886) and the two-way treatment by cultivar interaction (P = 0.5660) (Table 3.4). The glyphosate mean regrowth root biomass was the smallest at 90 mg plant^{-1}, while the non-treated mean was the largest at 158 mg plant^{-1} (Table 3.5). Even though the LSD was 73 mg plant^{-1}, it was concluded that the diclofop-methyl application mean was within 16% of the non-treated control (Table 3.5). This conclusion suggests that there is variable control of Italian ryegrass with diclofop-methyl

similar to results presented by Chandi et al. (2011). The conclusions of the glyphosate (Kuk and Burgos 2007) and pyroxsulam (Wells 2008) applications on regrowth root biomass correspond with previous research on controlling Italian ryegrass populations.

Data analysis of the regrowth root biomass indicated that there were differences between the twenty-six cultivars (P = 0.0001) across the POST herbicides (Table 3.4) with an LSD of 52 mg plant^{-1} (Table 3.6). The mean regrowth root biomass ranged from 82 to 171 mg plant^{-1}. The smallest and largest root biomass means belonged to the 'Winterhawk' and 'Big Boss Tetraploid Annual' cultivars, respectively (Table 3.6).

Differences in cultivar response to the herbicide application scenarios across the data measurements was the result of genotypic variations such as ploidy level (Inoue et al. 2014) and maturation timing (Redfearn et al. 2002). In addition, the stability or continual relative performance across years and locations of some ryegrass cultivars can vary between excellent to poor (Anonymous 2017; Redfearn et al. 2005). The stability factor varies because it depends upon the cultivar and its response to the surrounding environment (Redfearn et al. 2005). With respect to newer ryegrass cultivars, specific yield-limiting traits such as disease resistance, winter hardiness, nutritional value, etc. (Inoue et al. 2014) have been targeted for development over yield improvement (Redfearn et al. 2005). Therefore, this focused development could affect the overall biomass and yield potential of the currently available cultivars.

2016. Data analysis on initial, fresh shoot biomass indicated that there were no differences in treatment response (P = 0.2820) or the two-way treatment by cultivar interaction (P = 0.9929) for PRE herbicides flufenacet plus metribuzin or pyroxasulfone and their respective non-treated controls (Table 3.1). Regardless of the LSD of 4267 mg exp unit^{-1}, there were differences in the shoot biomass means between the treatments. The non-treated control had the

largest mean biomass at 2252 mg exp unit^{-1}, whereas the flufenacet plus metribuzin and pyroxasulfone means were 63 and 71 mg exp unit^{-1}, respectively (Table 3.2). These findings concur with previous research that pyroxasulfone controlled 83 to 100% of Italian ryegrass over two years, while flufenacet plus metribuzin applications provided 80% or greater control of Italian ryegrass (Grey and Bridges 2003; Ritter and Menbere 2002).

Likewise, the data analysis determined that there were no differences between the fifty-nine Italian ryegrass cultivars and their responses to the PRE treatments (P = 0.9946) (Table 3.1). However with an LSD of 736 mg exp unit^{-1}, there were shoot biomass differences between the cultivars similar to those for the 2015 experiments. The mean shoot biomass was the largest for the 'BAR LM 14167-4' cultivar at 1938 mg exp unit^{-1} and the smallest for the 'Wax Marshall' cultivar at 586 mg exp unit^{-1} (Table 3.7). The differences between shoot biomass correspond with the previously discussed UGA Statewide Variety Testing performance research on commercial Italian ryegrass cultivars (Anonymous 2017).

The analysis of the initial, fresh shoot biomass exhibited no differences in treatment variable response with P = 0.1058, but the two-way treatment by cultivar interaction was significant (P < 0.0001) across the diclofop-methyl, glyphosate, pinoxaden, or pyroxsulam applications and their respective non-treated controls (Table 3.8). For the treatment variable with an LSD of 1.46 g exp unit^{-1}, the pinoxaden application had the smallest mean shoot biomass at 0.40 g exp unit^{-1}, while the non-treated control mean had the largest at 2.31 g exp unit^{-1} (Table 3.9). Previous research on Italian ryegrass populations documented similar control of ryegrass with glyphosate (Kuk and Burgos 2007). These conclusions differ from experiments conducted

by Kuk and Burgos (2007) and Ellis et al. (2008) that lack of control of ryegrass by diclofop-methyl and pinoxaden applications occurred. However, lack of Italian ryegrass control with an application of pyroxsulam was not recorded which concurs with previous findings by Wells (2008).

The analysis concluded that there was a significant cultivar response ($P < 0.0001$) when cultivars were combined across treatments for the shoot biomass (Table 3.8). With an LSD of 0.21 g exp unit^{-1}, the commercial cultivars had a mean range of 0.29 to 1.70 g exp unit^{-1}. The 'BAR LM 15426' cultivar and the 'FLC4X' cultivar had the largest and smallest biomasses, respectively, for the POST treatments (Table 3.10).

For the regrowth shoot emergence data of the POST treatments and their respective non-treated controls, there were significant differences for the two-way treatment by cultivar interaction with $P = 0.0030$ (Table 3.8). However, there were no differences for the treatment response ($P = 0.1629$) (Table 3.8) which had an LSD of 15 shoots (Table 3.9). The smallest and largest mean shoot emergence counts were 3 plants for the pinoxaden and 20 plants for the non-treated control, respectively (Table 3.9). These results are similar to those recorded for the initial, fresh shoot biomass and for previous research that documented 90+ % control of Italian ryegrass with pinoxaden (Hofer et al. 2006). A closer analysis of the treatments displayed that the shoot emergence count differences between the diclofop-methyl, glyphosate, and pyroxsulam applications and the non-treated control were less than the LSD, which concurs with previous experiments indicating lack of control of Italian ryegrass populations by applications of diclofop-methyl (Chandi et al. 2011), glyphosate (Perez-Jones et al. 2005; Jasieniuk et al. 2008), and pyroxsulam (Salas et al. 2010).

There were significant differences for the cultivar variable ($P < 0.0001$) (Table 3.8) with an LSD of 3 plants for shoot emergence counts of the POST treatments (Table 3.10). The 'TAMTBO' cultivar has the smallest mean shoot emergence count at 3 plants. Meanwhile, the 'Becva' cultivar had the largest mean of 21 plants (Table 3.10).

Regrowth shoot biomass analysis for the POST treatments displayed no differences in treatment response ($P = 0.7065$), even though the two-way treatment by cultivar interaction was significant with $P < 0.0001$ (Table 3.8). The treatment LSD was determined to be 25 mg plant^{-1}, and the diclofop-methyl and non-treated applications had the smallest and largest mean shoot biomasses of 22 and 34 mg plant^{-1}, respectively (Table 3.9). These results contradict with research by Chandi et al. (2011) that documented cultivar control was variable when diclofop-methyl was applied. Pinoxaden and pyroxsulam application biomass means were within 15% of the non-treated control. Nonetheless, previous research conducted by Salas et al. (2010) that stated lack of control of ryegrass populations with pyroxsulam applications differed from the documented results. The regrowth shoot biomass data agrees with experiments in which glyphosate (Kuk and Burgos 2007) and pinoxaden (Hofer et al. 2006) applications controlled Italian ryegrass populations.

With $P < 0.0001$ (Table 3.8) and an LSD of 9 mg plant^{-1}, there were determined to be differences in cultivar response for the regrowth shoot biomass (Table 3.10). Regrowth shoot biomass means were the largest at 43 mg plant^{-1} for the 'GA102A' cultivar and the smallest for the 'GALM 1513' cultivar at 14 mg plant^{-1}. Sixty-nine percent of all cultivars were between 20 to 35 mg plant^{-1} (Table 3.10).

For the regrowth root biomass analysis of the POST treatments and their respective non-treated controls, no differences were recorded for the two-way treatment by cultivar interaction (P = 0.9688) or the treatment variable response (P = 0.2773) (Table 3.8). The glyphosate mean regrowth root biomass was the smallest at 77 mg plant^{-1}, while the pinoxaden and non-treated control means were the largest at 170 mg plant^{-1} (Table 3.9). With an LSD of 111 mg plant^{-1} (Table 3.9), it was concluded that the pinoxaden treatment lacked control of Italian ryegrass as previously documented in *Lolium rigidum* (Boutsalis et al. 2012). Parallel to the regrowth root biomass of the 2015 experiments, diclofop-methyl control of Italian ryegrass was variable amongst the cultivars which is similar to conclusions presented by Chandi et al. (2011). The glyphosate application's (Kuk and Burgos 2007) and the pyroxsulam application's (Wells 2008) control of the cultivars agree with previous research on controlling Italian ryegrass populations using these herbicides.

Data analysis of the regrowth root biomass (P = 0.0061) indicated that there were differences between the cultivars across the POST herbicides (Table 3.8) with an LSD of 90 mg plant^{-1} (Table 3.10). The mean regrowth root biomass ranged from 75 to 328 mg plant^{-1}. The smallest root biomass mean belonged to the 'FLC4X', whereas, the largest mean is associated with the 'ME94 Exp' cultivar (Table 3.10).

As previously discussed for the 2015 experiments, the differences in cultivar response across the data measurements for the herbicide application scenarios are due to genetic variations within the cultivars (Anonymous 2017; Inoue et al. 2014; Redfearn et al. 2002; Redfearn et al. 2005).

Conclusions

Greenhouse experiments demonstrated that control of commercially available Italian ryegrass cultivars was variable across experimental years and data measurements. For both experimental years, flufenacet plus metribuzin or pyroxasulfone control of the tested Italian ryegrass cultivars was in agreement with previously conducted research (Hulting et al. 2012; Grey and Bridges 2003; Ritter and Menbere 2002). Initial, fresh shoot biomass displayed that all cultivars tested were controlled by the diclofop-methyl, glyphosate, pinoxaden [2016], or pyroxsulam applications.

Regrowth data across all parameters exhibited differences in cultivar response to the POST herbicide applications. As shown by the shoot emergence counts, control of the tested ryegrass cultivars was lacking for the glyphosate application for the 2015 experiments. However, the 2016 experiments demonstrated a lack of control by diclofop-methyl, glyphosate, or pyroxsulam applications. For the shoot biomass data, diclofop-methyl and pyroxsulam lacked control of the Italian ryegrass cultivars tested for the 2015 experiments, whereas, all POST applications for the 2016 experiments controlled the tested cultivars. In the 2015 experiments, diclofop-methyl control was variable for the root biomass data, but all cultivars were considered controlled by the POST treatments. Yet in the 2016 experiments, there was a lack of control amongst the cultivars for the pinoxaden application.

A continuation of these experiments within the field setting could better determine the response of commercially available cultivars to small grain herbicides. Dose-responses evaluating additional rates of small grain herbicides should be completed. Further analyses on other commercially available Italian ryegrass cultivars could determine if lack of control to all small grain herbicides is developing in experimental seed lots.

Table 3.1: Analysis of variance for Cultivar and Treatment effects on 2015 and 2016 Georgia commercialized Italian ryegrass cultivars for flufenacet plus metribuzin or pyroxasulfone applications.[a]

Experiment	Effect	F-value	Pr >R	
2015[b]	Cultivar	0.85	0.6822	NS[d]
	Treatment	26.10	0.0002	***
	Treatment x Cultivar	0.75	0.8943	NS
2016[c]	Cultivar	0.58	0.9946	NS
	Treatment	1.99	0.2820	NS
	Treatment x Cultivar	0.68	0.9929	NS

[a]Initial, fresh shoot biomass data was combined across PRE herbicide treatments and their respective non-treated controls.
[b]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[c]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[d]Abbreviations: NS, not significant; * = level of probability at P = 0.05 to 0.01, respectively; ** = level of probability at P = 0.01 to 0.001, respectively; *** = level of probability at P < 0.001.

Table 3.2: Initial, fresh shoot biomass means of the 2015 and 2016 Georgia commercialized Italian ryegrass cultivars for the individual PRE treatments and the respective non-treated control.[a]

Experiment	Treatment	Mean
		mg exp unit^{-1}[d]
2015[b]	Flufenacet + metribuzin	75
	Pyroxasulfone	150
	NTC[e]	1623
LSD (0.05)		556
2016[c]	Flufenacet + metribuzin	63
	Pyroxasulfone	71
	NTC	2252
LSD (0.05)		4267

[a]Data was recorded 21 DAT (days after treatment) for each of the treatments.
[b]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[c]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[d]mg exp unit^{-1} = mg per experimental unit, a 237 mL cup (2015); a cell in plastic flat [51 cm x 26 cm x 6cm] (2016).
[e]Abbreviations: NTC, non-treated control.

Table 3.3: Initial, fresh shoot biomass mean for each of the 2015 Georgia commercialized Italian ryegrass cultivars 21 DAT[c] after PRE applications of flufenacet plus metribuzin or pyroxasulfone.[a]

Cultivar Name	_____mg exp unit^{-1}[b]_____
Andes (IS-LWT 13)	981
Attain Tetraploid Annual	1146
Big Boss Tetraploid Annual	1145
Credence (IS-LWT 14)	1301
Diamond T	1113
Earlyploid	1034
Flying A	743
Fria	840
GO-15-LN2	827
Grasshancer 100	1204
Jackson	682
Jumbo	735
KoSpeed Diploid Annual	770
KoWinearly Diploid Annual	698
Lonestar	737
Marshall	657
Maximus	1054
Meroa Tetraploid Italian	939
Nelson Tetraploid	1110
PS12	938
PS15	1188
Passerel Plus	720
Prine	708
TAMTBO	830
Tetrastar	787
Winterhawk	945
LSD (0.05)	377

[a]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses in SAS 9.4® were performed

[b]mg exp unit^{-1} = mg per experimental unit, or a 237 mL cup.

[c]Abbreviations: DAT, days after treatment.

Table 3.4: Analysis of variance for Cultivar and Treatment effects on 2015 Georgia commercialized Italian ryegrass cultivars for diclofop, glyphosate, or pyroxsulam applications. [a,b]

Variable	Effect	F-value	Pr >R	
Fresh shoot biomass	Cultivar	3.79	< 0.0001	***[c]
	Treatment	27.59	0.0110	**
	Treatment x Cultivar	1.81	0.0028	**
Regrowth shoot emergence	Cultivar	1.52	0.0755	NS
	Treatment	14.91	0.0262	*
	Treatment x Cultivar	1.04	0.4320	NS
Regrowth shoot biomass	Cultivar	0.91	0.5861	NS
	Treatment	0.50	0.7075	NS
	Treatment x Cultivar	1.04	0.4198	NS
Regrowth root biomass	Cultivar	2.82	0.0001	***
	Treatment	3.10	0.1886	NS
	Treatment x Cultivar	0.96	0.5660	NS

[a]Data measurements were combined across POST herbicide treatments and their respective non-treated controls.
[b]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[c]Abbreviations: NS, not significant; * = level of probability at P = 0.05 to 0.01, respectively; ** = level of probability at P = 0.01 to 0.001, respectively; *** = level of probability at P < 0.001.

Table 3.5: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the 2015 Georgia commercialized Italian ryegrass cultivars for the individual POST treatments and the respective non-treated control.[c]

Variable	Treatment	Mean
Fresh shoot biomass		g exp unit^{-1}[d]
	Diclofop-methyl	0.21
	Glyphosate	0.51
	Pyroxsulam	0.29
	NTC[g]	1.15
LSD (0.05)		0.37
Regrowth shoot emergence		#$^{-1}$[e]
	Diclofop-methyl	3
	Glyphosate	12
	Pyroxsulam	6
	NTC	19
LSD (0.05)		8
Regrowth shoot biomass		mg plant^{-1}[f]
	Diclofop-methyl	55
	Glyphosate	19
	Pyroxsulam	37
	NTC	27
LSD (0.05)		100
Regrowth root biomass		mg plant^{-1}[f]
	Diclofop-methyl	133
	Glyphosate	90
	Pyroxsulam	114
	NTC	158
LSD (0.05)		73

[a] Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the cultivars.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the cultivars.
[c]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses were performed.
[d]g exp unit^{-1} = g per experimental unit, or a 237 mL cup.
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.
[g]Abbreviations: NTC, non-treated control.

Table 3.6: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the 2015 Georgia commercialized Italian ryegrass cultivars for POST applications of diclofop, glyphosate, or pyroxsulam.[c]

Cultivar Name			Shoot	Root
	g exp unit^{-1}[d]	#$^{-1}$[e]	mg plant^{-1}[f]	
Andes (IS-LWT 13)	0.48	8	38	105
Attain Tetraploid Annual	0.61	10	34	160
Big Boss Tetraploid Annual	0.63	9	32	171
Credence (IS-LWT 14)	0.73	10	41	141
Diamond T	0.61	9	36	156
Earlyploid	0.66	11	34	149
Flying A	0.51	12	30	110
Fria	0.47	11	27	97
GO-15-LN2	0.42	12	28	91
Grasshancer 100	0.54	10	25	83
Jackson	0.41	10	50	96
Jumbo	0.53	7	46	180
KoSpeed Diploid Annual	0.48	11	32	109
KoWinearly Diploid Annual	0.41	10	24	83
Lonestar	0.52	12	38	126
Marshall	0.38	10	30	102
Maximus	0.60	10	25	139
Meroa Tetraploid Italian	0.56	9	33	87
Nelson Tetraploid	0.66	12	29	129
PS12	0.55	9	42	165
PS15	0.54	9	46	153
Passerel Plus	0.50	10	28	112
Prine	0.52	8	54	157
TAMTBO	0.57	10	40	138
Tetrastar	0.54	10	27	94
Winterhawk	0.41	10	22	82
LSD (0.05)	0.13	3	25	52

[a] Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the cultivars.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the cultivars.
[c]Locations were Athens and Tifton, GA conducted in 2015-2016. Twenty-six cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a 237 mL cup.
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.

Table 3.7: Initial, fresh shoot biomass mean for each of the 2016 Georgia commercialized Italian ryegrass cultivars 21 DAT[c] after PRE applications of flufenacet plus metribuzin or pyroxasulfone.[a]

Cultivar Name	_____mg exp unit^{-1}[b]_____
Andes (IS-LWT 13)	1102
Attain Tetraploid Annual	1247
BAR LM 14167-1	1699
BAR LM 14167-4	1938
BAR LM 15426	1161
BAR LM 16488	1264
BAR LM 16498	1670
Becva	1514
Big Boss Tetraploid Annual	1244
Credence	964
Diamond T	1290
Earlyploid	948
FL 4X Marmid	1015
FL 4X Marona	1449
FLAT-1	1139
FLAT-3	1786
FLC4X	1126
FLP166RB2X	1501
FLPE 2X	714
FLR164X	1312
FLRED 4X	1750
FLRSN4X	1742
Flying A	856
Fria	981
GA101M	1893
GA102A	933
GALM1401	1110
GALM1402	903
GALM1403	1191
GALM1501	1268
LSD (0.05)	736

[a]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[b]mg exp unit^{-1} = mg per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[c]Abbreviations: DAT, days after treatment.

Table 3.7 Ctd: Initial, fresh shoot biomass mean for each of the 2016 Georgia commercialized Italian ryegrass cultivars 21 DAT[c] after PRE applications of flufenacet plus metribuzin or pyroxasulfone.[a]

Cultivar Name	_____mg exp unit^{-1}[b]_____
GALM1502	1176
GALM1503	1322
GALM1513	882
GALM1514	1107
GALM1515	1012
Grazer	1061
Hostyn	1248
Jackson	924
Jumbo	887
Kodiak	1477
Lonestar Annual Ryegrass	1378
M2CVS Exp	910
ME4 Exp	842
ME94 Exp	1581
Maximus	1242
McKinley	1367
Nelson Tetraploid	1098
PS12	784
PS15	1022
Passerel Plus	907
Prine	1193
RMexp2013B	1407
SARG-FL	1152
Striker	1225
TAMTBO	1581
Tetrastar Tetraploid Annual Ryegrass	1573
Wax Marshall	586
Winterhawk	1140
WMWL Exp	1662
LSD (0.05)	736

[a]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4® were performed.
[b]mg exp unit^{-1} = mg per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[c]Abbreviations: DAT, days after treatment.

Table 3.8: Analysis of variance for Cultivar and Treatment effects on 2016 Georgia commercialized Italian ryegrass cultivars for diclofop, glyphosate, pinoxaden, or pyroxsulam applications. [a,b]

Variable	Effect	F-value	Pr >R	
Fresh shoot biomass	Cultivar	21.26	< 0.0001	***[c]
	Treatment	3.95	0.1058	NS
	Treatment x Cultivar	3.62	< 0.0001	***
Regrowth shoot emergence	Cultivar	10.42	< 0.0001	***
	Treatment	2.91	0.1629	NS
	Treatment x Cultivar	1.41	0.0030	**
Regrowth shoot biomass	Cultivar	4.97	< 0.0001	***
	Treatment	0.56	0.7065	NS
	Treatment x Cultivar	1.70	< 0.0001	***
Regrowth root biomass	Cultivar	1.61	0.0061	**
	Treatment	1.88	0.2773	NS
	Treatment x Cultivar	0.79	0.9688	NS

[a]Data measurements were combined across POST herbicide treatments and their respective non-treated controls.
[b]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4 ® were performed.
[c]Abbreviations: NS, not significant; * = level of probability at P = 0.05 to 0.01, respectively; ** = level of probability at P = 0.01 to 0.001, respectively; *** = level of probability at P < 0.001.

Table 3.9: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the 2016 Georgia commercialized Italian ryegrass cultivars for the individual POST treatments and the respective non-treated control.[c]

Variable	Treatment	Mean
Fresh shoot biomass		g exp unit^{-1}[d]
	Diclofop-methyl	0.74
	Glyphosate	0.94
	Pinoxaden	0.40
	Pyroxsulam	0.84
	NTC[g]	2.31
LSD (0.05)		1.46
Regrowth shoot emergence		#$^{-1}$[e]
	Diclofop-methyl	12
	Glyphosate	15
	Pinoxaden	3
	Pyroxsulam	17
	NTC	20
LSD (0.05)		15
Regrowth shoot biomass		mg plant^{-1}[f]
	Diclofop-methyl	22
	Glyphosate	23
	Pinoxaden	29
	Pyroxsulam	29
	NTC	34
LSD (0.05)		25
Regrowth root biomass		mg plant^{-1}[f]
	Diclofop-methyl	156
	Glyphosate	77
	Pinoxaden	170
	Pyroxsulam	139
	NTC	170
LSD (0.05)		111

[a] Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the cultivars.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the cultivars.
[c]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4 ® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.
[g]Abbreviations: NTC, non-treated control.

Table 3.10: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the 2016 Georgia commercialized Italian ryegrass cultivars for POST applications of diclofop, glyphosate, pinoxaden, or pyroxsulam.[c]

Cultivar Name			Shoot	Root
	g exp unit^{-1}[d]	__#$^{-1}$[e]__	_____mg plant^{-1}[f]_____	
Andes (IS-LWT 13)	0.58	12	16	108
Attain Tetraploid Annual	1.36	18	41	212
BAR LM 14167-1	1.22	16	34	144
BAR LM 14167-4	1.10	15	38	178
BAR LM 15426	1.70	14	31	189
BAR LM 16488	1.12	18	37	180
BAR LM 16498	1.27	16	33	136
Becva	0.99	21	37	170
Big Boss Tetraploid Annual	1.35	19	38	192
Credence	1.28	17	22	106
Diamond T	1.15	16	24	133
Earlyploid	1.23	18	33	147
FL 4X Marmid	0.99	14	32	121
FL 4X Marona	0.89	18	27	101
FLAT-1	0.68	16	29	118
FLAT-3	0.47	16	20	97
FLC4X	0.29	14	18	75
FLP166RB2X	0.60	9	19	97
FLPE 2X	0.56	14	23	115
FLR164X	0.79	16	31	114
FLRED 4X	0.73	16	21	101
FLRSN4X45	0.34	13	24	105
Flying A	1.01	15	35	141
Fria	0.88	8	21	131
GA101M	1.40	8	25	140
GA102A	1.01	6	43	155
GALM1401	0.79	14	18	122
GALM1402	1.18	11	25	114
GALM1403	1.40	14	34	166
GALM1501	0.70	13	23	87
GALM1502	0.94	14	31	88
GALM1503	0.68	17	22	108
LSD (0.05)	0.21	3	9	90

[a]Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the cultivars.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the cultivars.
[c]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4 ® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.

Table 3.10 Ctd: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the 2016 Georgia commercialized Italian ryegrass cultivars for POST applications of diclofop, glyphosate, pinoxaden, or pyroxsulam.[c]

Cultivar Name			Shoot	Root
	g exp unit^{-1}[d]	#$^{-1}$[e]	mg plant^{-1}[f]	
GALM1513	0.60	16	14	91
GALM1514	0.89	10	29	168
GALM1515	0.66	14	20	103
Grazer	1.11	13	19	106
Hostyn	0.95	12	28	163
Jackson	1.05	13	22	159
Jumbo	1.00	7	23	190
Kodiak	1.25	17	20	113
Lonestar Annual Ryegrass	0.99	14	22	148
M2CVS Exp	0.74	10	29	152
ME4 Exp	0.66	13	18	112
ME94 Exp	1.54	11	28	328
Maximus	1.25	15	26	134
McKinley	1.37	13	24	158
Nelson Tetraploid	1.37	14	30	198
PS12	1.48	11	34	160
PS15	1.38	10	39	170
Passerel Plus	0.99	18	25	149
Prine	1.30	20	33	199
RMexp2013B	0.96	13	29	154
SARG-FL	1.55	15	28	139
Striker	1.38	13	35	165
TAMTBO	1.41	3	36	190
Tetrastar Tetraploid Annual Ryegrass	1.65	6	36	156
Wax Marshall	0.63	11	23	148
Winterhawk	1.21	8	22	135
WMWL Exp	1.54	6	23	125
LSD (0.05)	0.21	3	9	90

[a]Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the cultivars.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the cultivars.
[c]Location was Tifton, GA conducted in 2016-2017. Fifty-nine cultivars were used. MIXED model analyses in SAS 9.4 ® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.

CHAPTER 4

HERBICIDE EFFECTS ON ITALIAN RYEGRASS [*LOLIUM PERENNE* L. SSP. *MULTIFLORUM* (LAM.) HUSNOT] CONTROL: FARM-COLLECTED BIOTYPE TRIALS[2]

[2] Simmons, D.B., T.L. Grey, W.K. Vencill, and A.S. Culpepper. To be submitted to *Weed Technology*.

Abstract

Greenhouse experiments were conducted from winter 2016 to spring 2017 in Tifton, Georgia, to better understand the distribution of herbicide resistant Italian ryegrass in small grain production across Georgia. Samples from 51 small grain production fields were taken during the fall of 2016; 34 of these samples consisted of high quality seed and were studied. Pyroxasulfone or flufenacet plus metribuzin treatments were applied to Italian ryegrass biotypes PRE at 59 and 477 g ai ha^{-1}, respectively. Diclofop-methyl (841 g ai ha^{-1}), glyphosate (875 g ae ha^{-1}), or pinoxaden (61 g ai ha^{-1}), or pyroxsulam (19 g ai ha^{-1}) were POST applied to ryegrass biotypes at Feekes' stage 1.0 (1 to 2 leaf). All farm-collected biotypes were controlled by the applications of pyroxasulfone or flufenacet plus metribuzin. Initial biomass displayed that all biotypes were controlled by the POST applied herbicides. Data analysis demonstrated that a lack of control in Italian ryegrass biotypes has occurred for the glyphosate and pyroxsulam applications for the regrowth shoot emergence counts and regrowth shoot biomass, respectively. A continuation of these experiments within the field setting would better determine the response of GA farm-collected biotypes to small grain herbicides, and further analyses should be conducted to determine if lack of control is developing in currently available populations.

Nomenclature: Diclofop-methyl; flufenacet plus metribuzin; glyphosate; pinoxaden; pyroxasulfone; pyroxsulam; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot

Key Words: Farm-collected biotypes, greenhouse experiments, ryegrass control, small grain production, small grain herbicides

Introduction

Originally from Europe (Lacefield et al. 2003), Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is listed as the fourth most common and most troublesome weed in Georgia small grain production (Webster 2012). Italian ryegrass is an erect winter annual grass species that can infest both winter and spring cropping systems (Lacefield et al. 2003; SARE 2007), particularly those in the southeastern United States (Betts et al. 1992). Due to the adaptability and extensive root structure of Italian ryegrass (Bryson and DeFelice 2009; SARE 2007; Ball et al. 1995), this species is distributed throughout all temperatures and soil types within the US especially those found in temperate regions (USDA NRCS 2017).

Italian ryegrass can grow to 1.3 m in height, allowing this species to protrude above wheat canopies (Bryson and DeFelice 2009; USDA NRCS 2017). As a result of this protrusion, light uptake by the crop is reduced allowing Italian ryegrass to have a greater leaf production rate (Ball et al. 1995). Previous experiments documented that wheat yield loss was attributed to decreased crop tillering due to the density of Italian ryegrass (Appleby et al. 1976; Leibl and Worhsam 1987), which becomes a stronger competitor when densities are at or above 150 plants m^{-2} (Acciaresi et al. 2001). However, semi-dwarf wheat cultivars such as 'Nugaines' and 'Hyslop' can have yield reductions from 37 to 39% when ryegrass densities are less than 100 plants m^{-2} (Appleby et al. 1976).

A mature Italian ryegrass plant can produce numerous tillers extending from 4.0 to 10.0 cm wide and 6.0 to 36.0 cm long with long, clasping auricles. As an obligate out-crosser (Lacefield et al. 2003; Inoue et al. 2014), multiple seed heads with awned seeds [102,000 seed kg^{-1}] (Lacefield et al. 2003) are produced by ryegrass plants (Bryson and DeFelice 2009).

Italian ryegrass can be found infesting small grain cropping systems throughout Georgia, where it can reduce yield by 4.2% for every 10 m^{-2} (Liebl and Worsham 1987). Previous experiments by Appleby et al. (1976) documented that wheat production can decrease by 60% as densities of Italian ryegrass increase.

In order to decrease ryegrass populations, herbicide applications are an efficacious and economical management method (Appleby et al. 1976; Justice et al. 1994). However, the consecutive application of herbicides to control Italian ryegrass has created instances where ryegrass populations seem to escape one or more of these herbicide applications. These populations are suspected to be herbicide resistant, or possess the heritable ability to survive and reproduce after an application of herbicide that normally would have been fatal to all wild type individuals (Johnson et al. 2009; Vencill et al. 2012). Previous research on collected ryegrass biotypes (Heap 2017) has confirmed Italian ryegrass resistance to mechanisms of action such as acetolactate synthase [ALS] (Kuk et al. 2008), acetyl Co-A carboxylase [ACCase] (Stanger and Appleby 1989), and 5-enolpyruvlshikimate-3-phosphate synthase [EPSPS] (Bond et al. 2014).

Several instances of Italian ryegrass resistance display how small grain producers generated herbicide resistance scenarios by alternating and overusing one chemistry after the other. When diclofop-methyl was first registered in 1982 for wild oat and annual grass [ryegrass] control in wheat and barley (EPA 2000), it was reported to have 90+ % control and applications resulted in higher yields than non-treated areas (Kirkland and O'Sullivan 1984; Brewster et al. 1977). However the repeated use of the ACCase-inhibiting herbicide diclofop-methyl, has resulted in resistant Italian ryegrass biotypes (Eleni et al. 2000; Kuk et al. 2008). As diclofop resistance increased, other chemistries such as ALS-inhibiting herbicides mesosulfuron-methyl [2004] (EPA 2004) and pyroxsulam [2008] (EPA 2008) became viable control options for Italian

ryegrass populations in wheat (Crooks and York 2002; Wells 2008). Yet, resistance and cross-resistance to these ALS chemistries quickly developed soon after registration and release (Kuk and Burgos 2007; Ellis et al. 2008; Salas et al. 2010; Chandi et al. 2011). Likewise, pinoxaden was thought to be an outstanding ACCase-inhibiting herbicide (Hofer et al. 2006) that was registered for grass control [ryegrass] in wheat in 2005 (EPA 2005). This herbicide was documented to control *Lolium* species by 90+ % (Hofer et al. 2006), but within a few years of its release, resistance had developed in Arkansas populations (Kuk et al. 2008) and Tennessee populations (Ellis et al. 2010).

In Georgia Italian ryegrass populations, resistance to diclofop-methyl [ACCase], mesosulfuron-methyl [ALS], and sethoxydim [ACCase] have been confirmed (Heap 2017). Due to multiple resistance cases already documented, there is a limited amount of chemistry that is recommended for Italian ryegrass control in small grains. Among the list herbicides that could be applied are diclofop-methyl, flufenacet plus metribuzin, flumioxazin plus pyroxasulfone, mesosulfuron-methyl, pinoxaden, pyroxasulfone, and pyroxsulam (Culpepper 2017). Of the herbicides recommended, many have similar mechanisms of action to those already confirmed for resistance (Culpepper 2017). Therefore, some ryegrass populations could have reduced control or total lack of control. Since limited herbicide screenings have been conducted on Georgia Italian ryegrass accessions, the purpose of this research is to determine the response of currently available Italian ryegrass populations to small grain herbicides.

Materials and Methods

Seed Collection. Seeds of Italian ryegrass were collected from 51 small grain production farms in 12 Georgia counties in the spring of 2016 (Table 4.1). In cooperation with University of Georgia Extension Agents, ryegrass samples of mature seed heads escaping the herbicide system were harvested by hand. To collect samples, researchers walked random tracts in zig-zag motions across the collection fields. Collected samples came from representative areas so that the diversity of the ryegrass population within that field was well characterized. Ryegrass seed heads were snapped at the meeting point of the inflorescence and the stem. Depending on the size of the field and time constraints, multiple large, brown paper bags were collected per field. Each paper bag was filled ½-way to full. Hand-harvested samples were stored at 20 C for 3 to 4 wk to allow adequate drying time before thrashing.

After drying, ryegrass inflorescences were hand-thrashed and then placed into a motor-driven thrasher (UGA)[3]. Seeds were separated from the chaff using a series of 20 gauge aluminum sieve plates [0.33 m diameter x 0.06 m deep] (Seedburo Equipment Company ®)[4]. Cleaned seeds were separated into mature and immature seeds using an Almaco Seed Cleaner with forced air (Allan Machine Company ®)[5]. Once the thrashing process was complete, seeds of each accession were collected and stored at 6 C until tested. To ensure adequate germination, seed were allowed to acclimate to 20 C over a 7 d period prior to planting.

Viability Experiment. A percent viability experiment was conducted on the fifty-one farm-collected biotypes prior to evaluation. Twenty seed from each field sample were sown into plastic flats [51cm x 26cm x 6cm] filled with steam-sterilized Tifton loamy sand [90% sand, 6% silt, 4% clay; fine-loamy, kaolinitic, thermic Plinthic Kandiudults] with pH 5.6 and 1.0% organic matter. Five days after planting [DAP] the number of emerged ryegrass seedlings were counted

allowing the calculation for percent seed germination. This experiment was replicated twice and the average percent viability for each farm-collected biotype was used in determining which biotypes were used in greenhouse experiments (Table 4.2).

Seventeen cultivars displayed poor germination of 10 to 50%. It is believed these samples were not fully mature when harvested and were discarded, with the exception of 'MoC1' to 'MoC3'. Thirty-four samples of farm-collected ryegrass seed were used in the greenhouse experiment.

Greenhouse Experiment. A greenhouse experiment was conducted twice from winter 2016 to spring 2017 at the Coastal Plains Experiment Station, Tifton, Georgia. For PRE treatments, ryegrass seeds were sown into 18 cell plastic flats [51cm x 26cm x 6cm] filled with steam-sterilized Tifton loamy sand [90% sand, 6% silt, 4% clay; fine-loamy, kaolinitic, thermic Plinthic Kandiudults] with pH 5.6 and 1.0% organic matter. For the POST treatments, seeds were sown into similar flats filled with a 1:1 ratio of steam-sterilized Tifton loamy sand to potting soil [45 to 55% composted bark fines, reed sedge, peat, other composited wood products and perlite][2]. Adding potting soil allowed for less soil leaching and increased soil retention in the flats, which ensured that all POST herbicide chemistry applied was readily available for uptake. Due to the influence of organic matter on the chemistries of PRE herbicides, it was not recommended that potting soil be added as it reduces the efficacy potential of the herbicides.

For all experiments, a teaspoon was used to sprinkle 30 to 40 seed of each biotype over the soil surface within each of the 18 cells of the flat. A quarter inch of the respected soil was placed on top of the sown seeds within each cell. Each teaspoon of seed had an average weight of 0.15 g. This measurement was used to ensure similar amounts of seed were planted into each cell within the flat regardless of the germination percentage.

Pyroxasulfone or flufenacet plus metribuzin at 59 and 477 g ai ha^{-1}, respectively, were applied PRE within 0 to 1 d of planting. Diclofop-methyl (841 g ai ha^{-1}), glyphosate (875 g ae ha^{-1}), pinoxaden (61 g ai ha^{-1}), or pyroxsulam (19 g ai ha^{-1}) were applied to emerged ryegrass at Feekes' stage 1.0 (Wise et al. 2011) or 7 d after planting. Recommended adjuvants COC (1% v/v) and UAN (2% v/v) were included in the pyroxsulam application. Herbicide rates corresponded to the recommendations for labeled uses in wheat [*Triticum aestivum* L.] cropping systems (Culpepper 2017). All herbicide application rates were made with a compressed air spray chamber delivering 187 L ha^{-1} volume of water at 138 kPa at 3 kph using a XR Teejet 11002 VS nozzle. A non-treated control for each cultivar and each herbicide application scenario [PRE vs. POST] was included in the experiment.

All plants within the greenhouse were fertilized weekly with 24-8-16 Miracle-Gro liquid fertilizer[1] and watered once a day for 5 minutes until field capacity. Supplemental light of 500 µmol m^{-2} s^{-1} light intensity was provided when necessary, and heaters kept the temperature between 25 to 29 C. These maintenance steps were taken to ensure that any symptomology, displayed by the Italian ryegrass seedlings, was due to the efficacy of the herbicide and not environmental stressors [i.e. lack of water, nutrients, etc.].

For locations receiving the PRE treatments, ryegrass seedlings that emerged after application were harvested 21 DAT by clipping the shoots at soil level and taking a fresh weight biomass measurement. All collected samples were placed into a drier at 95 C for 72 hours, after which dry weight biomass measurements were recorded.

For the POST herbicide treatments, all above-ground biomass was harvested 21 DAT by clipping all necrotic and non-necrotic shoots at soil level. A fresh weight biomass measurement was taken for each sample. All collected samples were placed into a drier at 95 C for 72 hours. Dry weight biomass measurements were then recorded for each of the samples.

Following the initial 21 DAT harvest, Italian ryegrass seedlings of POST treatments were allowed to regrow for 14 d. After this regrowth period, stand counts or counts of emerged shoots in each individual cell were recorded. Emerged shoots were harvested and biomass data was collected using similar methodology from the initial harvest at 21 DAT. Roots from each cell were also washed free of soil and drained of excess water. Each of the root samples had a fresh weight biomass measurement recorded before placing them into a drier at 95 C for 72 hours, after which dry weight biomass measurements were documented.

Experimental Design

There was one site location for the 2016 farm-collected biotypes. Greenhouse experiments were arranged in a split-plot design with two replications of treatments. Within the split-plot arrangement, the herbicide treatments represent the whole plot and the biotypes represent the subplot. Experiments were repeated twice from the winter of 2016 to the spring of 2017.

Analyses of all recorded data was combined across experiments and replications and subjected to a mixed-model analysis with an alpha of $P \leq 0.05$ in SAS 9.4 ®. Initial, fresh shoot biomass obtained from all PRE treatments and the respective non-treated controls was analyzed via a PROC GLM procedure. Additionally, initial, fresh shoot biomass and regrowth shoot emergence, fresh shoot biomass, and fresh root biomass data recorded for all POST treatments

and the respective non-treated controls were analyzed via a PROC GLM procedure. For the PROC GLM procedure, the main effects were the herbicide treatments and ryegrass biotypes, the two-way interaction of interest was the herbicide treatment by biotype, and the error term was determined to be the two-way interaction of herbicide treatment by experiment. This analysis procedure was chosen due to the large number of biotypes tested per experiment.

Results and Discussion

Upon analysis, data indicated that the two-way interaction of treatment by biotype varied. There were significant and non-significant interactions for treatment by biotype across the recorded measurements. For this reason, the main effects of treatment and cultivar and the two-way interaction of treatment by cultivar P-values are documented. Dry biomass data will not be presented for these experiments because the trends in data correspond to those displayed by the fresh biomass data.

Data analysis on initial, fresh shoot biomass indicated that there were no differences in treatment response ($P = 0.4112$) or the two-way treatment by biotype interaction ($P = 0.9085$) for PRE herbicides flufenacet plus metribuzin or pyroxasulfone and their respective non-treated controls (Table 4.3). Although the LSD was 1162 mg exp unit^{-1}, the non-treated control had the largest mean at 766 mg exp unit^{-1}, while flufenacet plus metribuzin and pyroxasulfone had shoot biomass means of 21 and 72 mg exp unit^{-1}, respectively (Table 4.4). These findings concur with previous research that pyroxasulfone controlled 83 to 100% of Italian ryegrass over two years, while flufenacet plus metribuzin controlled 100% of all Italian ryegrass (Hulting et al. 2012).

Analysis of the biotype response (P = 0.8853) to the PRE treatments displayed that there were no differences between the thirty-four biotypes when combined across treatments (Table 4.3). Yet with an LSD of 333 mg exp unit^{-1}, data displayed that there were differences in shoot biomass between the farm-collected Italian ryegrass biotypes. Mean shoot biomass across biotypes ranged from 82 to 728 mg exp unit^{-1} with the 'JC2' biotype and the 'WC5' biotype having the smallest and largest biomasses, respectively (Table 4.5). Differences in biotype response are a result of genetic variations that are known to develop in Italian ryegrass populations (Inoue et al. 2014).

Across the POST treatments of diclofop-methyl, glyphosate, pinoxaden, or pyroxsulam and their respective non-treated controls, the analysis of the initial, fresh shoot biomass displayed that there were significant differences in treatment variable response with P = 0.0022 (Table 4.6) and an LSD of 0.19 g exp unit^{-1} (Table 4.7). However, there were no differences for the two-way treatment by biotype interaction with P = 0.1027 (Table 4.6). For the treatment variable, the pinoxaden application had the smallest mean shoot biomass at 0.31 g exp unit^{-1}, whereas the non-treated control's mean was the largest at 1.05 g exp unit^{-1} (Table 4.7). Previous research on Italian ryegrass accessions indicated similar control of ryegrass with glyphosate (Kuk and Burgos 2007) and pinoxaden (Hofer et al. 2006) applications, but differ from previous experiments conducted by Chandi et al. (2011) and Salas et al. (2010) on variable and decreased control of Italian ryegrass with diclofop-methyl and pyroxsulam, respectively.

The analysis concluded that there was a significant biotype response ($P < 0.0001$) when biotypes were combined across treatments for the shoot biomass (Table 4.6). With an LSD of 0.20 g exp unit^{-1}, the farm-collected biotypes had a mean range of 0.15 to 1.02 g exp unit^{-1}. Similar to the results of the PRE experiments, the 'JC2' biotype had the smallest biomass but the 'WC7' biotype had the largest biomass for the POST treatments (Table 4.8).

For the regrowth shoot emergence data of the POST treatments and their respective non-treated controls, there were differences for the two-way treatment by biotype interaction ($P < 0.0001$) and the treatment response ($P = 0.0337$) (Table 4.6) which had an LSD of 6 shoots (Table 4.7). The largest and smallest mean shoot emergence counts were 14 plants for the glyphosate treatment and 3 plants for the pinoxaden treatment, respectively (Table 4.7). On closer analysis of the treatments, the shoot emergence counts for glyphosate were larger than the non-treated control at 13 plants, which concurs with previous experiments indicating lack of control of Italian ryegrass populations by applications of glyphosate (Perez-Jones et al. 2005; Jasieniuk et al. 2008) (Table 4.7). Also, these results concur with previous experiments that indicated high levels of control of Italian ryegrass populations with a pinoxaden application (Hofer et al. 2006). Results differ however from research conducted by Salas et al. (2010) that documented lack of control by pyroxsulam and research by Chandi et al. (2011) that stated ryegrass control was variable when diclofop-methyl was applied.

There were differences for the biotype variable ($P < 0.0001$) for shoot emergence counts of the POST treatments (Table 4.6). For this variable, the LSD was 4 plants between the biotypes. The 'JC2' biotype had the smallest mean shoot emergence count at 3 plants, while the 'WC1' biotype had the largest mean of 16 plants (Table 4.8).

Analysis on the regrowth shoot biomass for the POST treatments displayed that there were no differences in treatment response (P = 0.8460) or the two-way treatment by biotype interaction with P = 0.8971 (Table 4.6). Even though the treatment LSD was determined to be 39 mg plant^{-1}, the pyroxsulam application had mean shoot biomass of 31 mg plant^{-1} which was larger than the non-treated control mean of 30 mg plant^{-1} (Table 4.7). This data suggested that the pyroxsulam application lacked control of the Italian ryegrass biotypes which would concur with previously conducted experiments using this herbicide (Kuk and Burgos 2007; Salas et al. 2010). The collected data contradicted previous research that documented the variability in control of Italian ryegrass after applications of diclofop-methyl (Chandi et al. 2011). However, the regrowth shoot biomass data agrees with experiments in which glyphosate (Kuk and Burgos 2007) and pinoxaden (Hofer et al. 2006) applications controlled Italian ryegrass populations.

With P = 0.0001, there were determined to be significant differences in biotype response for the regrowth shoot biomass (Table 4.6). Regrowth shoot biomass means were the largest at 52 mg plant^{-1} for the 'JC3' biotype and the smallest for the 'WC4' and 'WC9' biotypes at 13 mg plant^{-1}. With respect to the regrowth shoot biomass means, the LSD was 17 mg plant^{-1} (Table 4.8).

The regrowth root biomass analysis, of the POST treatments and their respective non-treated controls, resulted in no differences for the two-way treatment by biotype interaction (P = 0.7162) or the treatment variable response (P = 0.5466) (Table 4.6). The glyphosate mean regrowth root biomass was the smallest at 98 mg plant^{-1}, while the non-treated control mean was the largest at 194 mg plant^{-1}. With an LSD of 154 mg plant^{-1} (Table 4.7), it was concluded that the diclofop-methyl application mean was within 8% of the non-treated control (Table 3.5).

This conclusion suggests that there is variable control of Italian ryegrass with diclofop-methyl similar to results presented by Chandi et al. (2011). The conclusions of the glyphosate (Kuk and Burgos 2007), pinoxaden (Hofer et al. 2006), and pyroxsulam (Wells 2008) applications on regrowth root biomass correspond with previous research on controlling Italian ryegrass populations.

Data analysis of the regrowth root biomass ($P = 0.0085$) indicated that there were differences between the thirty-four biotypes across the POST herbicides (Table 4.6) with an LSD of 89 mg plant^{-1} (Table 4.8). The mean regrowth shoot biomass ranged from 52 to 229 mg plant^{-1}. These smallest and largest root biomass means belonged to the 'JC2' and 'WC5', respectively (Table 4.8). These conclusions are similar to those reported for the initial, fresh shoot biomass for the PRE treatments.

Varying shoot biomass between biotypes and herbicide application scenarios is due to the highly heterozygous nature of Italian ryegrass (Inoue et al. 2014). Through allogamous reproduction, each individual within the Italian ryegrass population characterizes a different genotype. However, inbreeding of ryegrass populations can lead to a loss of fertility and vigor overtime (Inoue et al. 2014). Previous research has concluded that perennial ryegrass (*Lolium perenne* L.) populations from different ecotypes [locations] had differences in biomass and overall yield potential (Widdup and Ryan 1992). Although not confirmed, this could be similar to occurrences in Italian ryegrass biotypes.

Conclusions

Experiments demonstrated that control of Georgia farm-collected biotypes was variable across data measurements. Flufenacet plus metribuzin or pyroxasulfone control of the tested Italian ryegrass biotypes was in agreement with previously conducted research (Hulting et al. 2012; Grey and Bridges 2003; Ritter and Menbere 2002). Initial, fresh shoot biomass displayed that all farm-collected biotypes tested were controlled by the diclofop-methyl, glyphosate, pinoxaden, or pyroxsulam applications.

Regrowth data across all parameters exhibited differences in biotype response to the POST herbicide applications. As shown by the shoot emergence counts, control of the tested ryegrass biotypes was lacking for the glyphosate application. For the shoot biomass data, pyroxsulam lacked control of the farm-collected biotypes tested. Diclofop-methyl control was variable for the root biomass data, but all biotypes were considered to be controlled by the four POST treatments.

A continuation of these experiments in field settings is needed to determine if the documented results can be replicated. Dose-responses evaluating additional rates of small grain herbicides should be completed. Further analyses on other farm-collected biotypes in Georgia could determine if lack of control to all small grain herbicides is developing in currently available populations.

Table 4.1: GPS coordinates and herbicide use history of fields where Italian ryegrass biotypes originated.

Biotype Name	GPS Coordinates		County	Herbicide use history[a]
	°N	°W		
BC1	32.56	82.09	Burke	2,4-D; Gramoxone, 2,4-D
BC2	33.07	82.01	Burke	Prefix, Prowl, Roundup; Osprey, Harmony
BC3	32.56	82.08	Burke	Atrazine, Roundup, Gramoxone; Roundup, 2,4-D, Powerflex
BC4	32.56	82.07	Burke	Atrazine, Roundup, Gramoxone; Roundup, 2,4-D, Powerflex
DC1	32.16	83.45	Dooly	No Info; Zidua
DC2	32.08	83.51	Dooly	Glyphosate; Axial, Powerflex, Harmony
DC3	32.16	83.45	Dooly	No Info; Zidua
DC4	32.04	83.49	Dooly	No Info; Roundup + AMS, Gramoxone, 2,4-D
EC1	31.29	84.54	Early	No Info; 2,4-D
EC2	31.24	84.52	Early	No Info; 2,4-D
EC3	31.25	84.54	Early	No Info; 2,4-D
EC4	31.29	84.55	Early	No Info; 2,4-D
HC1	32.33	83.34	Houston	No Info; Pastora, Roundup
HC2	32.32	83.34	Houston	No Info
JC1	32.52	82.26	Jefferson	No Info; Powerflex
JC2	32.	82.	Jefferson	No Info; Roundup, Fierce
JC3	33.05	82.29	Jefferson	No Info
JG1	.	.	.	No Info
JG2	.	.	.	No Info
MC1	32.24	83.55	Macon	No Info; Axial, Harmony
MC2	32.18	84.06	Macon	No Info
MC3	32.24	84.05	Macon	No Info; 2,4-D, Harmony, Extra
MC4	32.26	83.56	Macon	No Info; Fierce, Harmony, Axial
MC5	32.21	83.56	Macon	No Info
MiC1	31.08	84.47	Miller	No Info; MCPA, Harmony
MiC2	31.08	84.44	Miller	No Info; MCPA, Harmony
MiC3	31.08	84.45	Miller	No Info; MCPA, Harmony
MiC4	31.11	84.41	Miller	No Info; MCPA, Harmony
MiC5	31.11	84.41	Miller	No Info; 2,4-D, Roundup
MoC1	33.44	83.30	Morgan	No Info; Axial, Powerflex
MoC2	33.36	83.28	Morgan	No Info
MoC3	33.40	83.32	Morgan	No Info
MoC4	33.40	83.32	Morgan	No Info; Harmony, Powerflex, Treflan, Axial
PC1	32.17	83.23	Pulaski	No Info; Fierce, 2,4-D, Harmony
PC2	32.18	83.35	Pulaski	No Info; Roundup, Gramoxone
RC1	31.75	84.73	Randolph	No Info; Axial
RC2	31.69	84.82	Randolph	No Info
RC3	31.68	84.72	Randolph	No Info; Axial
WC1	32.56	82.47	Washington	Axial, Harmony, Extra; Powerflex
WC2	32.58	82.52	Washington	Axial, Harmony, Extra; No Info
WC3	32.54	82.47	Washington	Axial, Harmony, Extra; Powerflex
WC4	32.52	82.48	Washington	No Info; 2,4-D
WC5	32.52	82.46	Washington	No Info; 2,4-D
WC6	32.54	82.47	Washington	No Info; 2,4-D
WC7	32.53	82.47	Washington	Powerflex; 2,4-D
WC8	32.54	82.44	Washington	Axial, Harmony, Extra; Powerflex
WC9	32.50	82.38	Washington	Axial, Harmony, Extra; Powerflex

[a]Herbicides used for Italian ryegrass control for 2015 and 2016 with the years separated by a semicolon.

Table 4.2: Percent viability of collected 2016 Georgia Italian ryegrass biotypes.

Biotype Name	Germination[a]
	%
BC1	50
BC2	75
BC3	50
BC4	25
DC1	75
DC2	80
DC3	75
DC4	40
EC1	90
EC2	50
EC3	40
EC4	50
HC1	90
HC2	60
JC1	50
JC2	40
JC3	75
JG1	100
JG2	100
MC1	50
MC2	50
MC3	50
MC4	40
MC5	90
MiC1	75
MiC2	90
MiC3	90
MiC4	90
MiC5	40
MoC1	25
MoC2	40
MoC3	50
MoC4	75
PC1	75
PC2	25
RC1	90
RC2	100
RC3	10
WC1	100
WC2	100
WC3	100
WC4	100
WC5	100
WC6	100
WC7	100
WC8	100
WC9	100

[a]Variability in germination is attributed to collection date and timing.
[b]For location of each biotype refer to Table 4.1.

Table 4.3: Analysis of variance for Cultivar and Treatment effects on Georgia farm-collected biotypes for flufenacet plus metribuzin or pyroxasulfone applications.[a,b]

Effect	F-value	Pr >R	
Cultivar	0.70	0.8853	NS[c]
Treatment	1.21	0.4112	NS
Treatment x Cultivar	0.73	0.9085	NS

[a]Initial, fresh shoot biomass data was combined across PRE herbicide treatments and their respective non-treated controls.
[b]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses were in SAS 9.4® were performed.
[c]Abbreviations: NS, not significant.

Table 4.4: Initial, fresh shoot biomass means of the Georgia farm-collected biotypes for the individual PRE treatments and the respective non-treated control.[a,b]

Treatment	Mean
	mg exp unit^{-1}[c]
Flufenacet + metribuzin	21
Pyroxasulfone	72
NTC[d]	766
LSD (0.05)	1162

[a]Data was recorded 21 DAT (days after treatment) for each of the treatments.
[b]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses in SAS 9.4® were performed.
[c]mg exp unit^{-1} = a cell in plastic flat [51 cm x 26 cm x 6cm].
[d]Abbreviations: NTC, non-treated control.

Table 4.5: Initial, fresh shoot biomass mean for each Georgia farm-collected biotype 21 DAT[c] after PRE applications of flufenacet plus metribuzin or pyroxasulfone .[a]

Biotype Name	_____mg exp unit^{-1}[b]_____
BC1	555
BC2	330
DC1	579
DC2	478
DC3	663
EC1	709
HC1	462
HC2	352
JC1	342
JC2	82
JC3	374
JG1	566
JG2	406
MC1	489
MC2	510
MiC1	345
MiC2	624
MiC3	505
MiC4	365
MoC1	183
MoC2	204
MoC3	256
MoC4	222
RC1	551
RC2	571
WC1	536
WC2	626
WC3	513
WC4	358
WC5	728
WC6	422
WC7	608
WC8	419
WC9	566
LSD(0.05)	333

[a]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses were in SAS 9.4® were performed.
[b]mg exp unit^{-1} = mg per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[c]Abbreviations: DAT, days after treatment.

Table 4.6: Analysis of variance for Cultivar and Treatment effects on Georgia farm-collected biotypes for diclofop-methyl, glyphosate, pinoxaden, or pyroxsulam applications.[a,b]

Variable	Effect	F-value	Pr >R	
Fresh shoot biomass	Cultivar	9.60	< 0.0001	***[c]
	Treatment	35.65	0.0022	**
	Treatment x Cultivar	1.23	0.1027	NS
Regrowth shoot emergence	Cultivar	7.50	< 0.0001	***
	Treatment	8.08	0.0337	*
	Treatment x Cultivar	2.00	< 0.0001	***
Regrowth shoot biomass	Cultivar	2.46	0.0001	***
	Treatment	0.33	0.8460	NS
	Treatment x Cultivar	0.81	0.8971	NS
Regrowth root biomass	Cultivar	1.81	0.0085	**
	Treatment	0.88	0.5466	NS
	Treatment x Cultivar	0.91	0.7162	NS

[a]Data measurements were combined across POST herbicide treatments and their respective non-treated controls.
[b]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses in SAS 9.4 ® were performed.
[c]Abbreviations: NS, not significant; * = level of probability at P = 0.05 to 0.01, respectively; ** = level of probability at P = 0.01 to 0.001, respectively; *** = level of probability at P < 0.001.

Table 4.7: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of the Georgia farm-collected biotypes for the individual POST treatments and the respective non-treated control.[c]

Variable	Treatment	Mean
Fresh shoot biomass		g exp unit^{-1}[d]
	Diclofop-methyl	0.35
	Glyphosate	0.54
	Pinoxaden	0.31
	Pyroxsulam	0.52
	NTC[g]	1.05
LSD(0.05)		0.19
Regrowth shoot emergence		#$^{-1}$[e]
	Diclofop-methyl	6
	Glyphosate	14
	Pinoxaden	3
	Pyroxsulam	8
	NTC	13
LSD(0.05)		6
Regrowth shoot biomass		mg plant^{-1}[f]
	Diclofop-methyl	26
	Glyphosate	20
	Pinoxaden	19
	Pyroxsulam	31
	NTC	30
LSD(0.05)		39
Regrowth root biomass		mg plant^{-1}[f]
	Diclofop-methyl	178
	Glyphosate	98
	Pinoxaden	143
	Pyroxsulam	158
	NTC	194
LSD(0.05)		154

[a]Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the biotypes.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the biotypes.
[c]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses in SAS 9.4® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.
[g]Abbreviations: NTC, non-treated control.

Table 4.8: Initial, fresh shoot biomass[a] and regrowth emergence and biomass[b] means of each Georgia farm-collected biotype for POST applications of diclofop, glyphosate, pinoxaden, or pyroxsulam.[c]

Biotype Name			Shoot	Root
	g exp unit^{-1}[d]	__#$^{-1}$[e]__	_____mg plant^{-1}[f]_____	
BC1	0.48	7	27	134
BC2	0.43	7	23	143
DC1	0.62	8	19	147
DC2	0.45	6	33	188
DC3	0.54	7	24	141
EC1	0.53	8	14	158
HC1	0.53	9	24	119
HC2	0.35	4	45	145
JC1	0.30	5	35	125
JC2	0.15	3	29	52
JC3	0.35	5	52	248
JG1	0.83	13	24	167
JG2	0.69	13	22	153
MC1	0.65	9	22	121
MC2	0.39	6	20	120
MiC1	0.53	7	29	148
MiC2	0.46	11	17	111
MiC3	0.52	8	26	168
MiC4	0.45	7	20	79
MoC1	0.33	5	41	122
MoC2	0.27	4	39	86
MoC3	0.21	4	31	126
MoC4	0.30	5	34	148
RC1	0.54	9	28	209
RC2	0.59	12	25	147
WC1	0.93	16	18	176
WC2	0.93	15	20	156
WC3	0.84	11	18	209
WC4	0.64	10	13	197
WC5	0.70	12	14	229
WC6	0.70	12	17	177
WC7	1.02	14	17	216
WC8	0.73	13	17	177
WC9	0.91	14	13	188
LSD(0.05)	0.20	4	17	89

[a]Initial, fresh biomass data was recorded 21 DAT (days after treatment) for each of the biotypes.
[b]Regrowth shoot emergence counts, fresh shoot biomass, and fresh root biomass data was recorded 35 DAT (days after treatment) for each of the biotypes.
[c]Location was Tifton, GA conducted in 2016-2017. Thirty-four biotypes were used. MIXED model analyses in SAS 9.4® were performed.
[d]g exp unit^{-1} = g per experimental unit, or a cell in plastic flat [51 cm x 26 cm x 6cm].
[e]# = number of plants.
[f]mg plant^{-1} = mg per ryegrass plant.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Results from preliminary greenhouse experiments indicate that a lack of control, in Georgia commercialized Italian ryegrass cultivars and farm-collected biotypes, has occurred for small grain herbicides. Initial, fresh shoot biomass and regrowth emergence and fresh biomass data exhibited that some of the tested Italian ryegrass populations had decreased control for acetyl Co-A carboxylase [ACCase]-, acetolactate synthase [ALS]-, and 5-enolpyruvlshikimate-3-phosphate synthase [EPSPS]-inhibiting herbicides. Since these mechanisms of action are prominent components of Italian ryegrass control programs in Georgia small grains, changes to these programs may be warranted. However, no lack of control was documented for the very-long-chain-fatty acid [VLCFA] or photosystem II [PSII] mechanisms of action.

Continuations of this study are needed to replicate these results in the field setting before any Georgia commercialized cultivars or farm-collected biotypes are classified as a potential issue. Further research is needed to test all commonly used herbicides in small grain production systems as well as non-chemical Italian ryegrass control options. It is suggested that suppliers should consider testing experimental Italian ryegrass cultivars for commercial use against all labeled herbicides to test the response of these cultivars. These assessments are needed to ensure that these cultivars are not inherently lacking control to the mechanisms of action which are currently being applied.

REFERENCES

[EPA] U.S. Environmental Protection Agency (2000) R.E.D. Facts: Diclofop-Methyl. https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-110902_1-Sep-00.pdf. Accessed: November 8, 2017

[EPA] U.S. Environmental Protection Agency (2004) Pesticide Fact Sheet: Mesosulfuron-methyl. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-122009_31-Mar-04.pdf. Accessed: November 8, 2017

[EPA] U.S. Environmental Protection Agency (2005) Pesticide Fact Sheet: Pinoxaden. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-147500_01-Jul-05.pdf. Accessed: November 8, 2017

[EPA] U.S. Environmental Protection Agency (2008) Pesticide Fact Sheet: Pyroxsulam. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-108702_27-Feb-08.pdf. Accessed: November 8, 2017

[OGTR] Office of the Gene Technology Regulator-Australian Government (2008) The Biology of *Lolium multiflorum* Lam. (Italian ryegrass), *Lolium perenne* L. (perennial ryegrass) and *Lolium arundinaceum* (Schreb.) Darbysh (tall fescue). Victoria, Australia: Department of Primary Industries, p 3

[SARE] Sustainable Agriculture Research and Education (2007) Annual ryegrass (*Lolium multiflorum*). Pages 74-77 *in* Clark A, ed. Managing Cover Crops Profitably. Maryland: United Book Press, Inc.

[USDA NRCS] U.S. Department of Agriculture Natural Resources Conservation Service (2017) Plant Fact Sheet: Italian Ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot]. Washington, DC: U.S. Department of Agriculture. 1 p

[USDA] U.S. Department of Agriculture (2012) U.S. Department of Agriculture Census: Specified Crops by Acres Harvested-2012. https://www.agcensus.usda.gov. Accessed: March 16, 2016

Acciaresi HA, Chidichimo HO, Sarandón SJ (2001) Traits related to competitive ability of wheat (*Triticum aestivum*) varieties against Italian ryegrass (*Lolium multiflorum*). Biol Agric and Hort 19:275-286

Anonymous (2015) Short-Term Ryegrass Flyer. http://www.seedforce.com.au/

Anonymous (2017) Georgis 2016-2017 Small Grain Performance Tests- Statewide Summary: Ryegrass Forage Performance, 2016-2017 with Two- and Three-Year Averages *Annual Publication 100-9*. Statewide Variety Testing, University of Georgia. Pp. 83-84

Appleby AP, Colbert DR, Olson PD (1976) Winter wheat yield reduction from interference by Italian ryegrass. Agro. J. 68:463-466

Ball DA, Klepper B, Rydrych DJ (1995) Comparative above-ground development rates for several annual grass weeds and cereal grains. Weed Sci 43:410-416

Beddows AR (1953) The ryegrasses in British agriculture: a survey. Welsh Plant Breeding Sta Bul, Ser H. 17:1-81

Betts KJ, Ehlke JE, Wyse DL, Gronwald JW, Somers DA (1992) Mechanism of inheritance of diclofop resistance in Italian ryegrass (*Lolium multiflorum*). Weed Sci 40:184-189

Blount AR, Prine GM (2016) Annual Ryegrass. http://edis.ifas.ufl.edu/ag104. Accessed August 29, 2017

Böger P, Matthes B, Schmalfuss J (2000) Towards the primary target of chloroacetamides-new finding pave the way. Pest Manag Sci 56:497-508

Bolland MDA, Rengel Z, Paszkudzka-Baizert L, Osborne LD (2001) Responses of subterranean clover and Italian ryegrass to application of lime. Austr Exp Agric 41:177-185

Bond JA, Eubank TW, Bond RC, Golden BR, Edwards HM (2014) Glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) control with fall-applied residual herbicides. Weed Technol 28:361-370

Boutsalis P, Gill GS, Preston C (2012) Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. Weed Technol 26:391-398

Brewster BD, Appleby AP, Spinner RL (1977) Control of Italian ryegrass and wild oats in winter wheat with HOE 23408. Agron J 69:911-913

Bryson CT, DeFelice MS, eds (2009) Weeds of the South. Athens, GA: University of Georgia Press. 390 p

Busi R, Gaines TA, Vila-Aiub MM, Powles SB (2014) Inheritance of evolved resistance to a novel herbicide (pyroxasulfone). Plant Science 217-218:127-134

Chandi A, York AC, Jordan DL, Beam JB (2011) Resistance to acetolactate synthase and acetyl Co-A carboxylase inhibitors in North Carolina Italian ryegrass (*Lolium perenne*). Weed Technol 25:659-666

Christoffers MJ (1999) Genetic aspects of herbicide-resistant weed management. Weed Technol 13:647-652

Cralle HT, Fojtasek TB, Carson KH, Chandler JM, Millet TD, Senseman SA, Bovey RW, Stone MJ (2003) Wheat and Italian ryegrass (*Lolium multiflorum*) competition as affected by phosphorous nutrition. Weed Sci 51:425-429

Crooks HL, York AC (2002) Italian ryegrass control in wheat with mesosulfuron-methyl. Pages 22 *in* Proceedings of the Southern Weed Science Society. Atlanta, GA: Southern Weed Science Society

Culpepper AS (2017) Small grain weed control. Pages 368-380 *in* Horton D, ed. Georgia Pest Management Handbook: 2017 Commercial Edition. 28th edn. Athens: University of Georgia Cooperative Extension

Dickson JW, Scott RC, Burgos NR, Salas RA, Smith KL (2011) Confirmation of glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) in Arkansas. Weed Technol 25:674-679

Eleni KS, Avi T, Baruch R (2000) Diclofop-resistant *Lolium rigidum* from northern Greece with cross-resistance to ACCase inhibitors and multiple resistance to chlorsulfuron. Pest Manag Sci 56:1054-1058

Ellis AT, Morgan GD, Mueller TC (2008) Mesosulfuron-resistant Italian ryegrass (*Lolium multiflorum*) biotype from Texas. Weed Technol 22:431-434

Ellis AT, Steckel LE, Main CL, De Melo MSC, West DR, Mueller TC (2010) A survey for diclofop-methyl resistance in Italian ryegrass from Tennessee and how to manage resistance in wheat. Weed Technol 24:303-309

Fernández-Moreno PT, Bastida F, Prado RD (2017) Evidence, mechanism and alternative chemical seedbank-level control of glyphosate resistance of a rigid ryegrass (*Lolium rigidum*) biotype from southern Spain. Fron Plant Sci 8: DOI:10.3389/fpls.2017.00450

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: A Unique Global Herbicide. Washington, DC: American Chemical Society Monograph 189, p 653

Grey TL, Bridges DC (2003) Alternatives to diclofop for the control of Italian ryegrass (*Lolium multiflorum*) in winter wheat (*Triticum aestivum*). Weed Technol 17:219-223

Grey TL, Newsom LJ (2017) Winter wheat response to weed control and residual herbicides. *in* Wanyera R, Owuoche J, eds. Wheat Improvement, Management and Utilization. DOI: 10.5772/63694

Hancock DW (2013) Top 10 Forages: At a Glance. University of Georgia Extension, University of Georgia. Pp 5-6

Hancock DW (2014) Annual Ryegrass. University of Georgia Extension, University of Georgia. 3 p

Hancock DW, Hicks R, Kichler JM, Smith RC (2015) Georgia Forages: Grass Species *Bulletin 1351*. University of Georgia Extension, University of Georgia. Pp 2-5

Heap I (2017) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: June 21, 2017

Hirschberg J, McIntosh L (1983). Molecular basis for herbicide resistance in *Amaranthus hybridus*. Science 222:1346-1349

Hively WD, Cox WJ (2001) Interseeding cover crops into soybean and subsequent corn yields. Agron J 93:308-313

Hofer U, Muehlebach M, Hole S, Zoschke A (2006) Pinoxaden-for broad spectrum grass weed management in cereal crops. J. of Plant Dis and Protec 0:989-995

Holtum JA, Powles PB (1991) Annual ryegrass: an abundance of resistance, a plethora of mechanisms. Proceedings of Brighton Crop Protection Conferences-Weeds. Farnham, Surrey, UK, p 1071-1077

Hulting AG, Dauer JT, Hinds-Cook B, Curtis D, Koepke-Hill RM, Mallory-Smith C (2012) Management of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) in Western Oregon with preemergence applications of pyroxasulfone in winter wheat. Weed Technol 26:230-235

Inoue M, Stewart AV, Cai H (2014) Italian ryegrass. Pages 36-53 *in* Cai H, Yamada T, Kole C, eds. Genetics, Genomics and Breeding of Forage Crops. Boca Raton: CRC Press

Jasieniuk M, Ahmad R, Sherwood AM, Firestone JL, Perez-Jones A, Lanini WT, Mallory-Smith C, Stednick Z (2008) Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: distribution, response to glyphosate, and molecular evidence for an altered target enzyme. Weed Sci 56:496-502

Jasieniuk M, Brule-babel AL, Morrison IN (1996) The evolution and genetics of herbicide resistance in weeds. Weed Sci 44:176-193

Jauhar PP (1993) Cytogenetics of the *Festuca-Lolium* complex. Berlin, Germany: Springer-Verlag Berlin Heidelberg. Pp 9-10

Johnson WG, Owen MDK, Kruger GR, Young BG, Shaw DR, Wilson RG, Wilcut JW, Jordan DL, Weller SC (2009) U.S. farmer awareness of glyphosate-resistant weeds and resistant management strategies. Weed Technol 23:308-312

Justice GG, Pepper TF, Solie JB, Epplin FM (1994) Net returns from Italian ryegrass (*Lolium multiflorum*) control in winter wheat (*Triticum aestivum*). Weed Technol 8:317-323

Kirkland KJ, O'Sullivan PA (1984) Control of wild oats in wheat with barban, diclofop methyl, flamprop methyl, and difenzoquat. Can J Plant Sci 64:1019-1021

Kobayashi M, Honda H, Yamaji Y, Hanai R (2007) Study on a new herbicide, KIH-485- the biological properties as a wheat herbicides. J Weed Sci. Tech 52:68-69

Kuk YI, Burgos NR (2007) Cross-resistance profile of mesosulfuron-methyl-resistant Italian ryegrass in the southern US. Pest Manag Sci 63:349-357

Kuk YI, Burgos NR, and Scott RC (2008) Resistance profile of diclofop-resistant Italian ryegrass (*Lolium multiflorum*) to ACCase- and ALS-inhibiting herbicides in Arkansas, USA. Weed Sci 56:614-623

Lacefield G, Collins M, Henning J, Phillips T, Rasnake M, Spitaleri R, Grogson D, Turner K (2003) Annual Ryegrass *AGR-179*. University of Kentucky Extension Service, University of Kentucky College of Agriculture. Pp 1-2

Liebl R, Worsham AD (1987) Interference of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*). Weed Sci 35:918-823

Mallory-Smith C, Campbell J, Hulting A, Thill D (2015) Managing herbicide-resistant weeds. Pages 1-6 in Peachey E, ed. Pacific Northwest Pest Management Handbook [online]. https://pnwhandbooks.org/weed/agronomic/cereal-grain-crops/spring-wheat. Accessed: August 14, 2017

McCullough P (2015) Annual Ryegrass Control in Hay Fields *Circular 1078*. University of Georgia Extension, University of Georgia. Pp 1-5

McCullough PE (2017) Weed control in grass pastures and hayfields. Pages 338-357 *in* Horton D, ed. Georgia Pest Management Handbook: 2017 Commercial Edition. 28th edn. Athens: University of Georgia Cooperative Extension

Michitte P, Espinoza N, De Prado R (2003) Cross-resistance to ACCase inhibitors of *Lolium multiflorum*, *Lolium perenne*, and *Lolium rigidum* found in Chile. Commun Agric Appl Biol Sci 68:397-402

Nandula VK, Reddy KN, Poston DH, Rimando AM, Duke SO (2008) Glyphosate tolerance mechanism in Italian ryegrass (*Lolium multiflorum*) from Mississippi. Weed Sci 56:344-349

Owen MDK, Zelaya IA (2005) Herbicide-resistant crops and weed-resistance to herbicides. Pest Manag Sci 61:301-311

Perez-Jones AK, Park KW, Colquhoun J, Mallory-Smith C, Shaner D (2005) Identification of glyphosate-resistant Italian ryegrass (Lolium multiflorum) in Oregon. Weed Sci 53:775-779

Porpiglia PJ, Watanabe A, Kurts M, Yamaji Y, Honda H (2006) KIH-485 potential in southern row crops. Pages 43 *in* Proceedings of the Southern Weed Science Society. San Antonio, TX: Southern Weed Science Society

Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C (1998) Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. 46:604-607

Powles SB, Preston C (1995) Herbicide cross resistance and multiple resistance in plants. http://hracglobal.com/files/Herbicide-Cross-Resistance-and-Multiple-Resistance-in-Plants.pdf Accessed: July 7, 2017

Powles SB, Preston C (2006) Evolved glyphosate resistance in plants: biochemical and genetic basis of resistance. Weed Technol 20:282-289

Preston C, Powles SB (2002) Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. Heredity 88:8-13

Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (Lolium multiflorum) in northern Idaho and eastern Washington. Weed Technol 24:281-288

Reddy KN (2001) Effects of cereal and legume cover crop residues on weeds, yield, and net return in soybean (*Glycine max*). Weed Technol 15:660-668

Redfearn DD, Venuto BC, Pitman WD, Alison MW, Ward JD (2002). Cultivar and environment effects on annual ryegrass forage yield, distribution, and nutritive value. Crop Sci 42: 2049-2054

Redfearn DD, Venuto BC, Pitman WD, Blouin DC, Alison MW (2005) Multilocation annual ryegrass cultivar performance over a twelve-year period. Crop Sci 45: 2388-2393

Ritter RL, Menbere H (2002) Preemergence control of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*). Weed Technol. 16:55-59

Romani M, Piano E, Pecetti L (2002) Collection and preliminary evaluation of native turfgrass accession in Italy. Genetic Resources and Crop Evol 49:341-348

Roux F, Reboud X (2007) Herbicide resistance dynamics in a spatially heterogeneous environment. Crop Protection 26:335-341

Salas RA, Burgos NR, Alcober EA, Lassiter R, Scott RC (2010) Multiple resistance to ACCase- and ALS-inhibitors in Italian ryegrass. Page 187 *in* Proceedings of the Southern Weed Science Society. Las Cruces, NM: Southern Weed Science Society

Salas RA, Burgos NR, Mauromoustakos A, Lassiter RB, Scott RC, Alcober EA (2013) Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. *multiflorum* in the United States. Journal of Crop and Weed 9:168-183

Shimabukuro RH (1990) Selectivity and mode of action pf the postemergence herbicide diclofop-methyl. Plant Growth Regulator Society of America Quarterly 18:37-54 ShowDocuments.aspx?DocumentID=1192. Accessed: August 29, 2017

Stanger CE, Appleby AP (1989) Italian ryegrass (*Lolium multiflorum*) accessions tolerant to diclofop. Weed Sci 37:350-352

Stebler FG, Volkart A (1913) Die besten Futterpflanzen. Erster Band, 4. Auflage. K.J. Wyss, Bern

Stone MJ, Cralle HT, Chnadler JM, Miller TD, Bovey RW, Carson KH (1998) Above – and below ground interference of wheat by Italian ryegrass. Weed Sci 46:438-441

Suvorova TN (1960) Italian ryegrass, *Lolium multiflorum*. Bul Appl Bot, Genet, and Plant Breeding 33:3-34.

Tanetani Y, Kaku K, Kawai K, Fujioka T, Shimizu T (2009) Action mechanism of a novel herbicide, pyroxasulfone. Pestic Biochem Phys 95:47-55

Tardif FJ, Holtum JAM, Powles SB (1993) Occurrence of a herbicide-resistant acetyl-coenzyme A carboxylase mutant in annual ryegrass (*Lolium rigidum*) selected by sethoxydim. Planta 1993:176-181

Taylor JM, Coats GE (1996) Identification of sulfometuron-resistant Italian ryegrass (*Lolium multiflorum*) selections. Weed Technol 10:943-946

Terrell EE (1968) A taxonomic revision of the genus *Lolium*: Technical Bulletin 1392. Washington, DC: U.S. Department of Agriculture, p 2

Vencill WK, ed (2002) Herbicide Handbook. 8th edn. Lawrence KS: Weed Science Society of America. Pp 136-137

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci Special Issue 60:2-30

Webster TM (2012) Weed Survey-Southern States: Grass Crops Subsection. Proceedings of the Southern Weed Science Society. Charleston, South Carolina, 64:351-352

Wells GS (2008) Pyroxsulam for broad-spectrum weed control in wheat. Pages 297- 299 *in* Proceedings of 16th Australian Weeds Conference. Brisbane, Queensland: Queensland Weeds Society

Wheeler DJB, Jacobs SWL, Whalley RDB (2002). Grasses of New South Wales. Armidale, Australia: University of New England Printery. Pp 1-446

Wheeler DL, Chappey C, Lash AE, Leipe DD, Madden TL, Schuler GD, Tatusova TA, Rapp BA (2000) Database resources of the National Center for Biotechnology Information. Nucleic

Widdup KH, Ryan DL (1992) Forage potential of wild populations of perennial ryegrass collected from southern New Zealand farms. Pages 161-165 *in* Proceedings of the New Zealand Grassland Association. Gore, New Zealand: New Zealand Grassland Association

Wilkins PW, Humphreys MO (2003) Progress in breeding perennial forage grasses for temperate agriculture. J Agric Sci 140:129-150

Wise K, Johnson B, Mansfield C, Krupke C (2011) Managing wheat by growth stage *ID-422*. Purdue Extension, Purdue University. Pp 1-5

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

Yu Q, Zhang XQ, Hashem A, Walsh MJ, Powles (2003) ALS gene proline (197) mutation confer ALS herbicide resistance in eight separated wild radish (*Raphanus raphanistrum*) populations. Weed Sci 51:831-838

SOURCE OF MATERIALS

[1]Miracle-Gro Fertilizer®, Scotts Miracle-Gro, Marysville, OH 43040

[2]Robin Hood Potting Soil®, Timber and Landscape Products Inc., Adel, GA 31620

[3]Thrasher, University of Georgia, Tifton, GA 31793

[4] Sieve Plates, Seedburo Equipment Company, Chicago, IL 60607

[5]Almaco Seed Cleaner®, Allan Machine Company, Ames, GA 50010

APPENDIX A

TRINEXAPAC-ETHYL WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVAR EVALUATIONS WITH VARIABLE RATES OF NITROGEN[3]

[3] Simmons, D.B., T.L. Grey, W. Faircloth, W.K. Vencill, and T.M. Webster. 2017. *Journal of Experimental Agriculture International*. 16:1-9.

Abstract

In the southeastern region of the United Sates, soft red winter wheat (*Triticum aestivum* L.) is grown in double-crop production systems. The plant growth regulator (PGR) trinexapac-ethyl is applied to improve wheat morphology by reducing height and increasing stem wall diameter, which can promote maximum yields. An experiment was conducted from 2014 to 2015 to evaluate the effects of trinexapac-ethyl with varying N rates on growth, lodging, and yield of five soft red winter wheat cultivars. Soft red winter wheat was treated with trinexapac-ethyl at 233 or 256 g ai ha-1at 60 d after planting, or as a split application of 128 g ai ha-1 60 and 108 d after planting. Nitrogen fertilizer at 112 or 168 kg ha-1 was applied at Feekes' stage 3-4. Crop heights, spike counts per m^2, and node length from the flag leaf to the base of the floral spike were collected 144 d after planting, with final yields, grain moisture, and test weight determined after harvest. There were no interactions for the main effects of PGR by nitrogen rate, PGR by cultivar, or cultivar by nitrogen rate. Trinexapac-ethyl at 256 at Feekes 4, or split applied at 128 g ha-1 at Feekes' 4 and 7, significantly reduced soft red winter wheat height, and distance from the flag-leaf node to base of the floral spike as compared to the non-treated control. While there were no yield differences for trinexapac-ethyl treatments, height reductions and improved stem strength would reduce lodging that can often lead to crop failure.

INDEX WORDS: N fertilizer, Plant growth regulators, Soft red winter wheat cultivars, Trinexapac-ethyl

Introduction

Soft red winter wheat (*Triticum aestivum* L.) is an autumn-seeded crop in the southeastern United States utilized for grain and forage. Soft red winter wheat is a secondary crop in multiple-cropping systems that can provide an economical way for farmers to produce two crops in one year [1,2]. From 2013 to 2016, 17 million ha year^{-1} of soft red winter wheat were harvested across the United States [3]. Due to the significance of wheat in many double-crop production systems, there has been a greater interest in wheat production practices, especially those that encourage high crop yield [4,5]. Primary requirements for high yields are uniform stand establishment followed by even tiller production which can be influenced by crop additives such as plant growth regulators and N fertilization [5].

Plant growth regulators (PGRs) are phytohormones that mimic or modify one or more specific physiological processes within a plant [6]. Organ development or photosynthetic properties are not negatively affected by PGRs but allow the crop to continue developmental sequence with retarded growth [7,8,9]. In small grain and forage production, PGRs reduce stem internodes and length (13-28%) and improve grain set, grain quality, development, and harvest ability [10,11,12,13]. PGRs have been reported to increase seed yields in tall fescue, creeping red fescue, perennial ryegrass, and annual ryegrass seed crops as much as 50% or greater [14,15,16,13,17]. Previous studies on Kentucky bluegrass displayed that trinexapac-ethyl reduced heat tolerance, total height, average clipping yield 30-45%, and growth rate but increased chlorophyll concentration [18,19,20,21].

Trinexapac-ethyl is a plant growth regulator for turf grass seed production and small grains in the southeast [22,15,10]. Trinexapac-ethyl is a unique Type II PGR in that it interferes with the production of gibberellins (GAs) later in gibberellin biosynthetic pathway with respect to other Type II PGRs [23,24,25]. Trinexapac-ethyl is an acyl cyclohexanedione derived from cyclohexanecarboxylate. It inhibits the hydroxylation of GA_{20} to the physiologically active GA_1 by inhibiting 3-β-hydroxylase, a regulatory enzyme [26,27,28]. The inhibition of this key enzyme prevents cell elongation (i.e. expansion) which causes a shortening of internodes, a strengthening of the stem, an increase in stem diameter, and reduction in lodging [11,29,13].

Trinexapac-ethyl applications interact with other management practices including canopy closure date, crop residue destruction, and nitrogen application [13,30,15,31]. There has always been interest in using timing and rate of N fertilizer application for intense soft red winter wheat management that maximize yield [5,32,33,34,35,36]. One study evaluated N uptake and use efficiency prior to and during the grain filling period in soft red winter wheat [37]. Another previous study noted that there was a strong correlation between grain protein/yield and N uptake under non-limiting N conditions [37]. Multiple research has shown that grain yield has been directly linked with the uptake of N [38,39,40,41].

University of Georgia recommendations for a N application are 90 to 112 kg ha^{-1} in a season for small grains production. In the autumn season, the cropping rotation strategy and previous crop can affect the amount of N fertilizer applied [42,43]. During the winter season, several applications of N can be made prior to stem elongation in wheat. Once tillers are being promoted, excessive N fertilization can lead to lodging and reductions in flour quality and

milling properties, so applications are not recommended after Feekes' growth stage 4-5 [44,42]. Soft red winter wheat cultivars are continuously changing due to breeding programs that seek to improve disease tolerance, quality, and grain yield [45,46,47,48,49].

There is limited information available on trinexapac-ethyl effects on wheat cropping systems in the southeastern US. Given variability in wheat cultivar response to plant growth regulators and N fertilization [50,51,38,52,37,36], a field study was designed to evaluate the effects of trinexapac-ethyl and varying N rates on winter wheat lodging and yield to five different wheat cultivars.

Materials and Methods

A field trial was conducted from 2014 to 2015 at the Southwest Georgia Branch Experiment Station (SWGREC) located in Plains Georgia (Latitude 32.036638; Longitude -84.397595). Soil type was a Faceville sandy loam (clayey, kaolinitic, thermic, Typic Kandiudults) with < 1% organic matter and pH of 6.1. The soil was conventionally prepared by disk harrowing, moldboard plowing 25 to 30 cm deep, then rotary tilling. Single plots were 1.8 m wide and 9.1 m long.

Soft red winter wheat cultivars AGS 2026, AGS 2060, Coker 9553, Coker 9700, and Cypress were sown at 323-377/m^2into seedbeds on 5 Dec 2014 at 101 kg ha^{-1} [53,54,55]. Higher seeding rates are not recommended for standard cultural practices because of the increased potential for disease and lodging [33]. Main effect of cultivar was blocked by replication. Within each cultivar block, N fertilizer rate and PGR applications were randomized.

Nitrogen fertilizer at 112 or 168 kg ha^{-1} was applied with a Gandy TM Spreader (The Gandy Co., Mankato, MN) on 22 Jan 2015 when wheat was in Feekes' growth stage 3-4. Trinexapac-ethyl was POST applied to wheat at 233 or 256 g ai ha^{-1} 60 d after planting, or as a split application of 128 g ai ha^{-1} each time at 60 and 108 d after planting. The PGR treatments were applied with a CO_2-pressurized broadcast sprayer with FF11002 nozzles calibrated to deliver 187 L ha^{-1} volume of water. Standard culture practices for wheat production were followed using University of Georgia recommendations for pest control [56].

At Feekes' stage 10.5 data for crop heights from soil to apex of the spike (cm), along with apical stem length from the flag-leaf node to the base of the floral spike (cm), was measured on 5 random plants for each variable. Data for spike counts per m^2 were also recorded. These data were collected 144 d after planting. Grain was harvested at Feekes' growth stage 11 after natural desiccation, using a small plot combine, grain was mechanically cleaned, and then for each plot grain moisture, yield, and test weight determined. Final grain yield was based on 13% moisture.

The experimental design was a three-way factorial arranged in a randomized complete block with four replications. Data was subjected to mixed-model ANOVA analysis in SAS 9.4®, and all two-way interactions were subjected to GLM procedures. Means were separated using an LSD at the $P = 0.05$ level.

Results and Discussion

The two-way interactions for crop height, head count, yield, and test weight for cultivar (C) x fertilizer (F), C x trinexapac-ethyl treatment (TE), and F x TE were not significant for any variable (Table 1). For flag-leaf to apex, C x F was significant (P = 0.0099), but not for C x TE or F x TE. As cultivars will vary for height due to genotype differences, the C x F difference is an indication of this variability [47]. Therefore, data for all variables for the main effects for TE were combined across N fertilizer treatment and cultivars, main effects of F were combined across TE and cultivars, and main effects of cultivars were combined across TE and F for analysis.

3.1 Trinexapac-ethyl: Wheat height varied amongst the four different PGR treatments. There were significant height reductions when TE was applied at 256 g ai ha^{-1}, and for the split application of 128 g ai ha^{-1} at Feekes' stage 4 and 7 (Table 2). Data indicated that the single TE application of 256 g ai ha^{-1} and split application of 128 g ai ha^{-1}, reduced plant heights to 80 and 76 cm, respectively as compared to the NTC with 83 cm. Similarly, there was a significant reduction in length of the stem for flag-leaf to the base of the floral apex, ranging from 9 to 11 cm, for any TE application treatment as compared to the NTC with 12 cm (Table 2). These findings correspond with previous research results that applications of trinexapac-ethyl at later wheat growth development stages reduce plant height [12]. There were no differences for any treatment for wheat head count per m^2 or grain test weight (Table 2). These results confer with previous research that reported PGR interactions were nonsignificant for test weight measurements [50].

Trinexapac-ethyl treatments had similar yields as compared to the non-treated control, 3,470 kg ha^{-1} and 3,660 kg ha^{-1}(Table 3). None of the TE treatments impacted these soft red winter wheat yields, indicating the crop safety for this PGR. It can be used to effectively reduce plant height, which could reduce potential for lodging. Previous research noted no differences in yield for TE applied to wheat in an experiment in South America, however they did not indicate the type of wheat [47]. Similar results have been reported with ethephon [50, 57].

3.2 N Fertilizer: Significant differences for wheat head count per m^2 were observed between the two N fertilizer applications. The 168 kg N ha^{-1} fertilizer treatment produced more wheat heads than the 112 kg N ha^{-1} application (Table 2). Previous research noted reduced spike number $plant^{-1}$ and grain number $spike^{-1}$ when lower nitrogen levels were present [36]. Crop height measurements displayed a similar response to the 112 and 168 kg N ha^{-1} applications in which the larger application rate produced a larger plant (Table 2). Previous research on dryland winter wheat indicated higher nitrogen rates result in larger plants with greater above-ground biomass than with lower application rates [58]. However, a taller height could potentially lead to lodging issues later in the growing season. With respect to crop height, the results differ from a previous study in which researchers reported no influence over wheat height due to nitrogen application rates [47]. For flag-leaf to the base of the floral apex measurements and grain test weights, no differences were detected between the 112 or 168 kg ha^{-1} treatments (Table 2).

For N application rates, there were significant differences in yield. The 168 kg N ha^{-1} application yielded 3,670 kg ha^{-1}, while the 112 kg N ha^{-1} rate yielded 3,440 kg ha^{-1} (Table 3). Previous research on soft red winter wheat indicated similar results and concluded that a positive

linear correlation exists between the increase in yield and increase in amount of N fertilizer applied [32]. More recent research noted that increases in nitrogen fertilizer applications had a positive influence on wheat grain protein and yield [36,59]. Furthermore, one researcher reported that a split fall and spring application of high nitrogen rates may be important for maximizing grain yield in soft red winter wheat [34].

3.3 Cultivar: Between all five cultivars, there were differences for each of the measurements recorded. With respect to wheat height, there were no differences between the Cypress and AGS 2026 cultivars but all other cultivars were significantly different (Table 2). AGS 2060 was the shortest of the cultivars at 75 cm while Coker 9553 was the tallest at 87 cm. However, previous research reported no difference in crop height across all seven winter wheat cultivars tested [50]. AGS2060, which had the smallest average with 382 heads, was the only significant cultivar for head count per m^2. All five cultivars were significantly different from one another for flag-leaf to the base of the floral apex measurements, with Cypress as the tallest and Coker 9700 as the shortest (Table 2). Wheat cultivar was the only significant ($P < 0.0001$) main effect observed for test weight. Amongst the cultivars, Coker 9700 and Cypress were statistically similar at 68 and 69 kg hL^{-1}. These cultivars differed from Coker 9553, AGS 2026, and AGS 2060 in which wheat cultivars AGS 2026 and Coker 9700 had the lowest and highest average test weights, respectively (Table 2).

With respect to yield, PGRs are designed to allow the full yield potential of wheat to be realized due to interfering with plant development [60]. It is the reduction in lodging of the cultivar that allows for the yield potential to be increased over other scenarios [61]. There were significant differences in yield among the cultivars, with Coker 9553 (3,290 kg ha^{-1}) having the

greatest yield and AGS 2060 having the least (2,960 kg ha^{-1}). Coker 9700 was similar to both AGS 2026 and Cypress, even though AGS 2026 yielded greater at 3,720 kg ha^{-1} (Table 3). Variable cultivar yield response corresponds with previous research using ethephon on seven winter wheat cultivars [50].

Conclusions

In conclusion, the split application of trinexapac-ethyl at Feekes' stage 4 and 7 significantly reduced overall stem length. While it did not improve wheat yield, trinexapac-ethyl could assist growers by preventing lodging from occurring which would improve harvest efficiency promoting greater yield. Each of the five cultivars had a varying degree of response to fertilizer and trinexapac-ethyl treatments. AGS 2060 had the least amount of response to all treatments and produced the lowest yield as compared to all other cultivars. Higher N fertilizer rates resulted in larger and higher yielding wheat as was expected. Future research will focus on repeating this study.

Appendix A.1: Analysis of variance for TE and N effects on wheat cultivars in Georgia[a]

Variable	Effect	F-value	Pr > R	
Crop height (cm)	Cultivar x Fertilizer	2.09	.11	NS[b]
	Cultivar x TE	1.46	.30	NS
	Fertilizer x TE	0.43	.79	NS
Flag-leaf to base of floral spike (cm)	Cultivar x Fertilizer	3.47	.01	**
	Cultivar x TE	0.83	.62	NS
	Fertilizer x TE	1.31	.27	NS
Head count/m^2	Cultivar x Fertilizer	1.90	.09	NS
	Cultivar x TE	1.19	.15	NS
	Fertilizer x TE	0.35	.73	NS
Test weight (kg hL^{-1})	Cultivar x Fertilizer	1.45	.22	NS
	Cultivar x TE	0.91	.54	NS
	Fertilizer x TE	0.44	.72	NS
Yield (kg ha^{-1})	Cultivar x Fertilizer	0.61	.65	NS
	Cultivar x TE	0.85	.63	NS
	Fertilizer x TE	0.25	.86	NS

[a]Location was Plains, GA conducted in 2014-2015. Cultivars were AGS 2026, AGS 2060, Coker 9553, Coker 9700, and Cypress. MIXED model analysis was performed.
[b]Abbreviation: NS, not significant; ** = level of probability at P = 0.01 to 0.001, respectively.

Appendix A.2: Crop height, head count, and flag-leaf to apex as influenced by cultivar, fertilizer application, and growth regulator application conducted for 2014-2015.[a]

Variable	Rate	Timing	Crop height		Flag-leaf to base of floral spike		Head count		Test weight[b]	
Main effect of growth regulator	g ha^{-1}	Feekes' stage	cm		cm		# m^2		kg hL^{-1}	
Nontreated			83	a	12	a	412	a	67	a
Trinexapac-ethyl	233	4	81	ab	11	b	423	a	67	a
Trinexapac-ethyl	256	4	80	b	11	b	425	a	66	a
Trinexapac-ethyl	128 + 128	4 + 7	76	c	9	c	415	a	67	a
Main effect of fertilizer	kg ha^{-1}									
Nitrogen fertilizer	112	3-4	79	b	11	a	408	b	67	a
Nitrogen fertilizer	168	3-4	81	a	11	a	430	a	67	a
Main effect of cultivar										
Coker 9553			87	a	12	b	420	a	67	b
Coker 9700			82	b	9	e	442	a	69	a
Cypress			78	c	13	a	427	a	68	a
AGS 2026			78	c	11	c	427	a	64	d
AGS 2060			75	d	10	d	382	b	65	c

[a]The two-way interactions of cultivar x fertilizer and fertilizer x trinexapac-ethyl were not significant; therefore, data were combined across variables. However, the two-way interaction of cultivar x fertilizer was significant for flag-leaf to base of floral spike measurements.

[b]Means with a variable followed by the same letter are not significant according to Fisher's protected LSD test at $P \leq 0.05$

Appendix A.3: Yield as influenced by cultivar, fertilizer treatments, and growth regulator applications conducted for 2014-2015.[a]

Variable	Rate	Yield[b]	
Main effect of growth regulator	g ha^{-1}	kg ha^{-1}	
Nontreated		3660	a
Trinexapac-ethyl	233	3570	a
Trinexapac-ethyl	256	3470	a
Trinexapac-ethyl	128 + 128	3520	a
Main effect of fertilizer	kg ha^{-1}		
Nitrogen fertilizer	112	3440	b
Nitrogen fertilizer	168	3670	a
Main effect of cultivar			
Coker 9553		3920	a
Coker 9700		3660	bc
Cypress		3590	c
AGS 2026		3720	b
AGS 2060		2960	d

[a]The two-way interactions of cultivar x fertilizer, fertilizer x trinexapac-ethyl, and cultivar x fertilizer were not significant for yield; therefore, data were combined across variables.
[b]Means with a variable followed by the same letter are not significant according to Fisher's protected LSD test at $P \leq 0.05$

REFERENCES

1. Grey, T.L. Utility of residual herbicides in no-till double-crop glyphosate resistant soybean production. Crop Manag. 2007. Accessed: February 6, 2017.Available: https://dl.sciencesocieties.org doi: 10.1094/CM-2007-0122-01-RS.
2. Moss JW, Tubbs RS, Grey TL, Smith NB, Johnson JW. Assessment of double-crop and relay-intercropping systems of peanut with soft red winter wheat and residual herbicides. Crop Forage Turfgrass Manage. 2017;*(In press)*.
3. [USDA] U.S. Department of Agriculture Wheat Data: Yearbook Tables. U.S. Department of Agriculture: Environmental Research Service. 2017. Accessed 9 February 2017. Available: https://www.ers.usda.gov/data-products/wheat-data/
4. Culpepper AS, York AC. Weed management in no-till bromoxynil-tolerant cotton (*Gossypium hirsutum*). Weed Tech. 1997;11(2):335-345.
5. Roth, GW, Marshall HG, Hatley OE, Hill Jr RR. Effect of management practices on grain yield, test weight, and lodging of soft red winter wheat. Agron. J. 1984;76:379-383
6. Mitchell JW, Rice RR. Plant-growth regulators. Pages 256-266 *in* Yearbook of Agriculture. USDA: United States Government Printing Office.1942.
7. Qian YL, Engelke MC, Foster MJV, Reynolds S. Trinexapac-ethyl restricts shoot growth and improves quality of Diamond zoysiagrass under shade. HortSci. 1998;33(6);1019-1022.
8. Stier JC, Rogers JN III, Flore JA. Nitrogen and trinexapac-ethyl effects on photosynthesis of Supina bluegrass in reduced light conditions. Agron. Abstr. Madison: ASA Publ.;1997.

9. Kaufmann JE. Understanding turfgrass growth regulation. In: Leslie AR, editor. Handbook of integrated pest management for turf and ornamentals. Boca Raton: CRC Press; 1994.
10. Rolston MP, Trethewey JAK, Chynoweth R, McCloy B. Trinexapac-ethyl delays lodging and increases seed yield in perennial ryegrass seed crops. N.Z. J. Agr. Res. 2010;53(4):403-406.
11. Marchese JA, Suchoronczek A, Civieiro JC, Ascari CL, Fedrigo K. Efecto del trinexapac-etilo asociado con la fertilización nitrogenada elevada y parcelada en la productividad del trigo BRS-220. Brazilian J. of Applied Tech. for Agr. Sci. 2016;9:105-111. Brazilian.
12. Borm GEL, van den Berg W. Effects of the application rate and time of the growth regulator trinexapac-ethyl in seed crops of *Lolium perenne* L. in relation to spring nitrogen rate. Field Crop Res. 2008;105(3):182-192.
13. Chastain TG, Young WC III, Silberstein TB, Garbacik CJ. Performance of trinexapac-ethyl on Lolium perenne seed crops in diverse lodging environments. Field Crop Res. 2014;157:65-70.
14. Chastain TG, Young WC III, Garbacik CJ, Silberstein TB. Trinexapac-ethyl rate and application timing effects on seed yield and yield components in tall fescue. Field Crop Res. 2015;173:8-13.
15. Zapiola ML, Chastain TG, Garbacik CJ, Silberstein TB, Young WC III. Trinexapac-ethyl and open field burning maximize seed yield in creeping red fescue. Agron. J. 2006;98(6):1427-1434.
16. Chynoweth RJ, Trethewey JAK, Rolston MP, McCloy BL. Reduced stem length increases perennial ryegrass seed yield. Agron. N.Z. 2014;44:61-70.

17. Trethewey JAK, Rolston MP, McCloy BL, Chynoweth RJ. The plant growth regulator, trinexapac-ethyl, increases seed yield in annual ryegrass (*Lolium multiflorum* Lam.). N.Z. J. Agric. Res. 2016;59(2):113-121.
18. Heckman NL, Horst GL, Gaussoin RE, Young LJ. Heat tolerance of Kentucky bluegrass as affected by trinexapac-ethyl. HortSci. 2001;36(2):365-367.
19. Heckman NL, Horst GL, Gaussoin RE, Tavener BT. Trinexapac-ethyl influence on cell membrane thermostability of Kentucky bluegrass leaf tissue. Scientia Horticulturae. 2002;92(2):183-186.
20. Beasley JS, Branham BE, Ortiz-Ribbing LM. Trinexapac-ethyl affects Kentucky bluegrass root architecture. HortSci. 2005;40(5):1539-1542.
21. Xiao-ying W, Tian-ming HU, Quan-zhen W, Li-min T, Xiao-ling Z, Kai T. Growth of Kentucky bluegrass as influenced by nitrogen and trinexapac-ethyl. Agr. Sci. 2009;8(12):1498-1502.
22. Anonymous. Palisade EC specimen label. Greensboro, NC: Syngenta Crop. 2017a. Available http://www.cdms.net/ldat/ld2P2008.pdf. Accessed February 2, 2017.
23. Watschke TL, DiPaola JM. Plant growth regulators. Golf Course Mgt. 1995;63:59-62.
24. Ervin EH, Koski AJ. Growth responses of *Lolium perenne* L. to trinexapac-ethyl. HortSci. 1998;33(7) :1200-1202.
25. Rademacher W. Gibberellins: metabolic pathways and inhibitors of biosynthesis. In: Boger P, Sandmann G, editors. Target sites of herbicide action. Boca Raton: CRC Press; 1989.
26. Rademacher W, Temple-Smith KE, Griggs DL, Hedden P. The mode of action of acylcyclo-hexanediones-a new type of growth retardant. Current Plant Sci. Biotech. in Agric. 1992;13:571-577.

27. Adams R, Kerber E, Pfister K, Weiler EW. Studies on the action of the new growth retardant CGA 163’935 (cimectacarb). In: Karssen CM, van Loon LC, Vreugdenhil D, editors. Progress in plant growth regulations. Amsterdam: Kluwer Academics; 1992.
28. Hedden P. Gibberellin biosynthesis enzymes and the regulation of gibberellin concentration. In: Takahashi N, Phinney BO, MacMillian J, editors. Gibberellins. New York: Springer-Verlag; 1991.
29. Stefen DLV, Souza CA, Coelho CMM, Gutkoski LC, Sangoi L. A adubação nitrogenada durante o espigamento melhora a qualidade industrial do trigo (Triticum aestivum cv. Mirante) cultivado com regulador de crescimento etil-trinexapac. Revista de la Facultad de Agronomía, La Plata. 2015;114(2):161-169. Brazilian.
30. Rolston MP, Trethewey JAK, Chynoweth RJ, McCloy BL. Italian ryegrass seed yield; trinexapac-ethyl and closing date interaction. Agron. N.Z. 2012;42:119-127.
31. Rolston MP, McLoy BL, Trethewey JAK. Nitrogen-moddus interaction in perennial ryegrass seed production. Agron. N.Z. 2007;37:37-44.
32. Boquet DJ, Johnson CC. Fertilizer effects on yield, grain composition, and foliar disease of doublecrop soft red winter wheat. Agron J. 1987;79(1):135-141.
33. Beuerlein JE, Oplinger ES, Reicosky D. Yield and agronomic characteristics of soft red winter wheat as influenced by management. J. Prod. Agric. 1991;4(1):124-131.
34. No Thesis Comparison. Vieira LV. Determination of optimum fall and spring nitrogen fertilizer rates for maximizing grain yield of soft red winter wheat sown at variable planting dates. University of Arkansas. 2016.
35. Hitz K, Clark AJ, Van Sanford DA. Identifying nitrogen-use efficient soft red winter wheat lines in high and low nitrogen environments. Field Crop Res. 2017;200:1-9.

36. Tamang BG, Brasier KG, Thomason WE, Griffey CA. Differential responses of grain yield, grain protein, and their associated traits to nitrogen supply in soft red winter wheat. J. Plant Nutr. Soil Sci. 2017. Accessed 3 March 2017. http://dx.doi.org/10.1002/jpln.201600312

37. Van Sanford DA, MacKown CT. Variation in nitrogen use efficiency among soft red winter wheat genotypes. Theor Appl Genet. 1986;72(2):58-163.

38. Austin RB, Ford MA, Edrich JA, Blackwell RD. The nitrogen economy of winter wheat. J Agri Sci. 1977;88(1):59-167.

39. Desai M, Bhatia CR. Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica. 1978;27(2):561-566.

40. Cox MC, Qualset CO, Rains DW. Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. Crop Sci. 1985;25(3):435-440.

41. Loffer CM, Rauch TL, Busch RH. Grain and plant protein relationships in hard red spring wheat. Crop Sci. 1985;25(3):521-524.

42. Harris, G. Wheat fertilization and liming. Pages 19-20 *in* Southern Small Grains: Resource Management Handbook. Athens: University of Georgia Press. 2013.

43. Hargrove WL, Touchton JT, Johnson JW. Previous crop influence on fertilizer nitrogen requirements for doublecropped wheat. Agron J. 1983;75(6):855-859.

44. Wise K, Johnson B, Mansfield C, Krupke C. Managing wheat by growth stage. Purdue Extension: Purdue University. 2011. Accessed 2 February 2017. https://www.extension.purdue.edu/extmedia/ID/ID-422.pdf

45. Liu S, Abate ZA, Lu H, Musket T, Davis GL, McKendry AL. QTL associated with Fusarium head blight resistance in the soft red winter wheat Ernie. Theor. and Applied Gen. 2007;115(3):417-427.

46. Hao Y, Chen Z, Wang Y, Bland D, Buck J, Brown-Guedira G, Johnson J. Characterization of a major QTL for adult plant resistance to stripe rust in US soft red winter wheat. Theor. and Applied Gen. 2011;123(8):1401-1411.

47. Benetoli da Silva TR, Schmidt R, Tavares da Silva CA, Nolla A, Favero F, Poletine JP. Effect of trinexapac-ethyl and nitrogen fertilization on wheat growth and yield. J. Food Agr. Env. 2011;9(3&4):596-598.

48. Campbell LG, Lafever HN. Cultivar x environment interactions in soft red winter wheat yield tests. Crop Sci. 1977;17(4):604-608.

49. McKendry AL, Tague DN, Miskin KE. Effect of 1BL.1RS on agronomic performance of soft red winter wheat. Crop Sci. 1996;36(4):844-847.

50. Wiersma DW, Oplinger ES, Guy SO. Environment and cultivar effects on winter wheat response to ethephon plant growth regulator. Agron. J. 1986;78(5):761-764.

51. Crook MJ, Ennos AR. The effect of nitrogen and growth regulators on stem and root characteristics associated with lodging in two cultivars of winter wheat. J. Exp. Bot. 1995;46(8):931-938.

52. Dubois JB, Fossati A. Influence of nitrogen uptake and nitrogen partitioning efficiency on grain yield and grain protein concentration of twelve winter wheat genotypes (*Triticum aestivum* L.) Z Pflanzenzucht. 1981;86:41-49.

53. Anonymous. AGS 2026. Georgia's Integrated Cultivar Release System. 2017b. Accessed 13 March 2017. Available: http://georgiacultivars.com/cultivars/wheat.

54. Anonymous. AGS 2060. Georgia Seed Development. 2017c. Accessed 13 March 2017. Available: http://gsdc.com/crops/.

55. Costa JM, Bockelman HE, Brown-Guedira G, Cambron SE, Chen X, Cooper A, Cowger C, Dong Y, Grybauskas A, Jin Y, Kolmer J, Murphy JP, Sneller C, Souza E. Registration of the soft red winter wheat germplasm MD01W233-06-1 resistant to fusarium head blight. J. Plant Reg. 2010;4(3):255-260.

56. Horton D, editor. Georgia Pest Management Handbook: 2017 Commercial Edition. 28th ed. Athens (GA): University of Georgia Cooperative Extension; 2017.

57. Cox WJ, Otis DJ. Growth and yield of winter wheat as influenced by chlormequat chloride and ethephon. Agron. J. 1989;81(2):264-270.

58. Nielsen DC, Halvorson AD. Nitrogen fertility influence on water stress and yield of winter wheat. Agron. J. 1991;83(6):1065-1070.

59. Pal M, Rao LS, Jain V, Srivastava AC, Pandey R, Raj A, Singh KP. Effect of elevated CO2 and nitrogen on wheat growth and photosynthesis. Biol. Plant. 2005;49(3):467-470.

60. Berti M, Zagonel J, Fernandes EC. Wheat varieties yield under trinexapac-ethyl and nitrogen rates. Scientia Agraria 2007;8:127-134.

61. Dahnous K, Vigue GT, Law AG, Konzak CF, Miller DG. Height and yield response of selected wheat, barley, and triticale cultivars of ethephon. Agron. J. 1982;74(3):580-582.