CHARACTERIZATION OF ACETYL COENZYME A INHIBITOR RESISTANCE IN TURFGRASS AND GRASSY WEEDS

by

TRENT MATTHEW TATE

(Under the Direction of Paul Raymer)

ABSTRACT

A herbicide resistance weed control system that utilizes naturally occurring mutations to ACCase inhibiting herbicides could greatly improve selectivity and control of grassy weeds in turf. Knowledge regarding the presence of these mutations in grasses is needed to guide development. This research surveyed 24 species of warm-season, cool-season, and grassy weed species for the presence of mutations at amino acid positions 1781, 1999, 2027, 2041, 2076, 2088, and 2096. These species were also subjected to three rates, 0, 400, and 1200 g a.i. ha^{-1} of fenoxaprop herbicide. Resistance mutations were found at position 1781 in three of 24 species surveyed (*Festuca ovina, Festuca rubra,* and *Poa annua*). Nine species were resistant to fenoxaprop (*Agrostis capillaris, Festuca ovina, Festuca rubra, Lolium multiflorum, Lolium perenne, Paspalum dilatatum, Poa annua, Zoysia japonica,* and *Zoysia matrella*). These results will aid in the development of an ACCase resistant system for turfgrass.

INDEX WORDS: Acetyl coenzyme A carboxylase, mutations, fenoxaprop, herbicide resistance.

CHARACTERIZATION OF ACETYL COENZYME A INHIBITOR RESISTANCE IN TURFGRASS AND GRASSY WEEDS

by

TRENT MATTHEW TATE

BSES, University of Georgia, 2009

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

2012

CHARACTERIZATION OF ACETYL COENZYME A INHIBITOR RESISTANCE IN TURFGRASS AND GRASSY WEEDS

by

TRENT MATTHEW TATE

Major Professor:	Paul Raymer
Committee:	Melanie Harrison-Dunn
	Patrick McCullough

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2012

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Turfgrass is utilized around the world as the primary cover for various entities such as airports, athletic fields, cemeteries, churches, commercial buildings, golf courses, home lawns, schools, parks, and roadsides. In Georgia alone, it is estimated the turfgrass encompasses over 1.6 million acres (Waltz et al., 2010). While turfgrass adds aesthetic value to a landscape, it also prevents soil erosion and reduces runoff from rainfall.

Turfgrass related industries also contribute significantly to our economy with the greatest contributions coming from the sod and seed industries and from turf maintenance activities. Maintenance activities include trimming, fertilization, pest control, disease control, and weed control (Waltz et al., 2010). Weed control can add significantly to maintenance costs especially in highly maintained turf.

Turfgrass weeds can be classified as either broadleaf weeds or grassy weeds. Broadleaf weeds have many selective herbicides registered for their control in turf and in general are more easily managed than grassy weeds. Currently there are few selective herbicides for the control of grassy weeds in turfgrass and they are often difficult or impossible to control in turf.

Herbicide selectivity could be introduced as a system for easier management of hard to control weeds. One well-known example of this is the introduction of Roundup Ready™ crops. In this system, resistance to glyphosate is engineered into the crop by inserting one or more foreign genes using biotechnology methods. This gene insertion

gives the host plant resistance to glyphosate which allows the grower to kill all other plants by spraying glyphosate without harming the crop. These systems have made weed control much more efficient. Crops that were developed using this kind of approach are commonly referred to as genetically modified (GM). There are many other herbicide resistance systems both GM and non-GM that have been used in row crops. Some examples other than Roundup Ready™ include glufosinate resistance (LibertyLink®) and imazamox resistance (Clearfield®). Glufosinate resistant crops are developed using genetic engineering by transforming the plant with the BAR gene which when transcribed by the plant gives the plant resistance to glufosinate. The BAR gene was isolated from a bacteria and was shown to be the source of resistance to glufosinate. When the BAR gene is transcribed an enzyme is produced that degrades glufosinate to a form that is not harmful to the plant (Gordon-Kamm et al., 1990). Imazamox is an acetolactate synthase inhibiting herbicide and resistance is derived from mutagenesis and selection. This system was developed in maize, wheat, rice, canola, and sunflower (Tan et al., 2005).

The technology necessary to develop herbicide resistance turfgrass using GM approaches is readily available and well documented. Herbicide resistant cultivars have been developed in various turfgrass species by inserting genes conferring resistance to various herbicides. Glufosinate resistance was introduced in creeping bentgrass (*Agrostis stolonifera* L.) (Kim et al., 2007), creeping bentgrass (*Agrostis palustris* Huds.) (Hartman et al., 1994), 'Tifeagle' bermudagrass, (*Cynodon dactylon* L. x *C. transvaalensis* Burtt-Davy) (Goldman et al., 2004), seashore paspalum (*Paspalum vaginatum* Sw.) (Kim et al., 2009), and zoysiagrass (*Zoysia japonica* Steud.) (Toyama et al., 2003) by inserting the BAR gene. The BAR gene when engineered into a plant's genome and transcribed gives

the host plant resistance to glufosinate. Glyphosate and glufosinate resistance were engineered into creeping bentgrass (*A. stolonifera*) (Lee et al., 2011) and acetolactate synthase (ALS) inhibitor resistance was engineered into tall fescue (*Festuca arundinacea* Schreb.) (Sato et al., 2009).

Attempts to commercialize a GM-based herbicide resistance system for enhanced weed control in a turfgrass species have thus far been unsuccessful due to environmental concerns and the high costs of deregulation. A non-GM-based herbicide resistance system for turfgrass could provide much improved weed control options and avoid concerns and costs associated with deregulation of GM-based systems. Currently, there are very few options for the removal of perennial grassy weeds from a turfgrass stand. For example, selective removal of common bermudagrass from a turfgrass species, such as seashore paspalum or hybrid bermudagrass, is not possible with currently available herbicides. Control of these weeds would be much more efficient and effective if a herbicide resistance weed control system was developed. The acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicides have potential for this type of application due to their ability to selectively control grass species.

Acetyl-coenzyme A carboxylase (EC.6.4.1.2) is a key enzyme involved in the production of fatty acids used in the formation of cell walls. ACCase catalyzes the first committed step in *de novo* fatty acid synthesis. The homomeric plastidic ACCase is the target for ACCase inhibiting herbicides in the monocotyledon Poaceae. In these species it is thought that the ACCase inhibiting herbicides competitively bind with the enzyme blocking the function of the enzyme as a catalyst of the carboxylation of acetyl-CoA to produce melonyl-CoA. In dicot species, the plastidic ACCase is multimeric and not

sensitive to ACCase inhibiting herbicides, therefore making ACCase inhibiting herbicides specific to the Poaceae family (Yu et al., 2007). There are three chemical groups of ACCase inhibiting herbicides, aryloxyphenoxypropionate (APP), cylcohexanedione (CHD), and phenylpyrazoline (PPZ). ACCase inhibiting herbicides were introduced in 1978 and are widely used to control grass species.

Resistance to ACCase inhibiting herbicides occurs naturally, and a total of 42 Poaceae species worldwide have been documented to have resistance to ACCase inhibiting herbicides (Heap, 2012). The two main resistance mechanisms to ACCase inhibitors are metabolic and site of action resistance. Metabolic resistance refers to the plant's ability to metabolize the herbicide to a less toxic or non-toxic form. Metabolic resistance to ACCase inhibiting herbicides is not well understood but normally provides only moderate levels of resistance. Metabolic resistance to ACCase inhibiting herbicides has been reported in barley, ryegrass, and wheat (Preston et al., 1996; Romano et al., 1993; Vila-Aiub et al., 2005).

The other type of resistance, known as site of action resistance or target site resistance, is conferred by the presence of single base pair mutations in the CT domain of the ACCase enzyme which is thought to affect the binding of ACCase inhibiting herbicides (Delye et al., 2005; Devine, 1997; Liu et al., 2007). In total, eight DNA substitutions that confer resistance to ACCase inhibiting herbicides have been identified in various Poaceae species at seven different amino acid positions of the CT domain of ACCase (Powles and Yu, 2010). Henceforth, these will be referred to as mutations sites. Each of these mutations is a single base pair substitution in the gene that encodes chloroplastic ACCase (Devine, 1997). The most commonly found resistance mutation is

the 1781 Ile to Leu change in the carboxyl transferase domain of the plastidic ACCase gene where the codon number is in reference to that of *Alopecurus myosuroides* Huds.. The other mutation sites include 1999 Trp to Cys, 2027 Trp to Cys, 2041 Ile to Asn, 2041 Ile to Val, 2078 Asp to Gly, 2088 Cys to Arg, and 2096 Gly to Ala (Delye et al., 2002).

Recently, resistance to the ACCase inhibiting herbicide sethoxydim was developed using *in vitro* selection for the 1781 Ile to Leu mutation in seashore paspalum (Heckart et al., 2010). In this method callus tissue was generated and subjected to the herbicide sethoxydim in the growth medium for a total of 63 days. Any callus tissue that grew on this herbicide-containing medium was regenerated and once regenerated those whole plants were tested for herbicide resistance and sequenced to confirm the presence of the 1781 Ile to Leu mutation.. This approach could be used to develop herbicide resistance that could improve control of problematic grassy weeds in other turf species. While these resistance mutations are known and have been documented in many Poaceae species, turfgrass species and their common grassy weeds have not been surveyed for these mutations. A better understanding of the presence of these mutations in turfgrass and weedy grass species is needed to develop of ACCase inhibitor resistance in turf.

There are many turfgrass species with moderate to high levels of tolerance to ACCase inhibiting herbicides and in some cases ACCase inhibiting herbicides are registered for use on them. Envoy Plus (Valent U.S.A. Corporation, Walnut Creek, CA) contains the active ingredient clethodim and is registered for use in centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) (Anonymous, 2007). Illoxan (Bayer Environmental Science, Research Triangle Park, NC) contains diclofop-methyl and is registered for use to control goosegrass (*Eleusine indica* L.) in bermudagrass on golf

courses (Anonymous, 2012). Acclaim Extra (Bayer Environmental Science, Research Triangle Park, NC) has the active ingredient fenoxaprop and is registered for use on Kentucky bluegrass (*Poa pratensis* L.), fine fescues (*Festuca ovina* L. and *F. rubra* L.), tall fescue (*Festuca arundinacea*), and zoysiagrass (*Zoysia japonica* and *Z. matrella* [L.] Merr.) (Anonymous, 2005). Fusilade II (Syngenta Professional Products, Greensboro, NC) contains the active ingredient fluazifop-butyl and is registered for use in tall fescue (*F. arundinacea*) and zoysiagrass (*Z. japonica* and *Z. matrella*) (Anonymous, 2009). Segment (BASF Specialty Products, Research Triangle Park, NC) contains sethoxydim and is registered for use in centipedegrass (*E. ophiuroides*) and fine fescues (*F. ovina* and *F. rubra*) (Anonymous, 2010). The nature of resistance to ACCase inhibiting herbicides for bermudagrass, centipedegrass, Kentucky bluegrass, tall fescue and zoysiagrass is not known. The high level of resistance in these species could possibly be due to the presence of a resistance conferring mutation at any of the seven amino acid positions known to confer ACCase inhibitor resistance. Before a herbicide resistance weed control system can be developed in turfgrass using ACCase inhibitors, research is warranted to identify mutations and different resistance spectrums to the ACCase inhibitors.

LITERATURE CITED

Anonymous. 2005. Acclaim Extra herbicide label, Bayer Environmental Science, Research Triangle Park, NC.

Anonymous. 2007. Envoy Plus herbicide label, Valent U.S.A. Corporation, Walnut Creek, CA.

Anonymous. 2009. Fusilade II herbicide label, Syngenta Crop Protection, Greensboro, NC.

Anonymous. 2010. Segment herbicide label, BASF Corporation, Research Triangle Park, NC.

Anonymous. 2012. Illoxan herbicide label, Bayer Environmental Science, Research Triangle Park, NC.

Delye C., Matejicek A., and Gasquez J. 2002. PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manage Sci. 58:474-478.

Delye C., Zhang X.Q., Michel S., Matejicek A., and Powles S.B. 2005. Molecular bases for sensitivity to acetyl-coenzyme a carboxylase inhibitors in black-grass. Plant Physiol. 137:794-806.

Devine M.D. 1997. Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibitors: a review. Pestic. Sci. 51:259-264.

Goldman J.J., Hanna W.W., Fleming G.H., and Ozias-Akins P. 2004. Ploidy variation among herbicide-resistant bermudagrass plants of cv. 'TifEagle' transformed with the bar gene. Plant Cell Rep. 22:553-560.

Gordon-Kamm, W.J., Spencer, T.M., Mangano, M.L., Adams, T.R., Daines, R.J., Start, W.G., O'Brien, J.V., Chambers, S.A., Adams, W.R., Willetts, N.G., Rice, T.B., Mackey, C.J., Krueger, R.W., Kausch, A.P., and Lemaux, P.G. 1990. Transformation of maize cells and regeneration of fertile transgenic plants. The Plant Cell 2:603-618.

Hartman C.L., Lee L., Day P.R., and Tumer N.E. 1994. Herbicide-resistant turfgrass (*Agrostis palustris* Huds.) by biolistic transformation. Bio/Technology. 12:919-923.

Heap I.M. 2012. International survey of herbicide-resistant weeds. Weed Science. http://www.weedscience.org (March 21, 2012)

Heckart D.L., Parrott W.A., and Raymer P.L. 2010. Obtaining sethoxydim resistance in seashore paspalum. Crop Sci. 50:2632-2640.

Kim K.-M., Song I.J., Lee H.-Y., Raymer P., Kim B.S., and Kim W. 2009. Development of seashore paspalum turfgrass with herbicide resistance. Korean J. Crop Soc., Korean Society Of Crop Science. 54:427-432.

Kim S.J., Lee J.Y., Kim Y.M., Yang S.S., Hwang O.J., Hong N.J., Kim K.M., Lee H.Y., Song P.S., and Kim J.I. 2007. Agrobacterium-mediated high-efficiency transformation of creeping bentgrass with herbicide resistance. Journal Plant Biol. 50:577-585.

Lee K.W., Kim K.Y., Kim K.H., Lee B.H., Kim J.S., and Lee S.H. 2011. Development of antibiotic marker-free creeping bentgrass resistance ag a.i.nst herbicides. Acta Biochimica Et Biophysica Sinica. 43:13-18.

Liu W.J., Harrison D.K., Chalupska D., Gornicki P., O'Donnell C.C., Adkins S.W., Haselkorn R., and Williams R.R. 2007. Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. Proc. Natl. Acad. Sci. USA. 104:3627-3632.

Powles S.B. and Yu Q. 2010. Evolution in action: plants resistant to herbicides. in: S. Merchant, et al. (Eds.), Annu. Rev. of Plant Biol. 61:317-347.

Preston C., Tardif F.J., Christopher J.T., and Powles S.B. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochem. Physiol. 54:123-134.

Romano M.L., Stephenson G.R., Tal A., and Hall J.C. 1993. The effect of monooxygenase and glutathione-S-tranferase inhibitors on the metabolism of diclofop-methyl and fenoxaprop-ethyl in barley and wheat. Pesticide Biochem. Physiol. 46:181-189.

Sato H., Takamizo T., Shimizu T., Kawai K., and Kaku K. 2009. Conferred resistance to an acetolactate synthase-inhibiting herbicide in transgenic tall fescue (*Festuca arundinacea* Schreb.). HortScience. 44:1254-1257.

Tan S., Evans R.R., Dahmer M.L., Singh B.K., and Shaner D.L. 2005. Imidazolinone-tolerant crops: history, current status and future. Pest Manage Sci. 61:246-257.

Toyama K., Bae C.H., Kang J.G., Lim Y.P., Adachi T., Riu K.Z., Song P.S., and Lee H.Y. 2003. Production of herbicide-tolerant zoysiagrass by Agrobacterium-mediated transformation. Mol. Cells. 16:19-27.

Vila-Aiub M.M., Neve P., and Powles S.B. 2005. Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. New Phytol. 167:787-796.

Waltz C., Murphy T., and Landry G. 2010 Turfgrass industry facts in Georgia. in: U. o. Georgia (Ed.), 2010 Turfgrass Pest Control.

Yu Q., Collavo A., Zheng M.Q., Owen M., Sattin M., and Powles S.B. 2007 Diversity of acetyl-coenzyme a carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. Plant Physiol. 145:547-558.

CHAPTER 2

SURVEY OF TURFGRASS AND COMMON GRASSY WEEDS FOR MUTATIONS CONFERRING RESISTANCE TO ACCASE INHIBITING HERBICIDES [1]

---

1 Trent M. Tate, Patrick E. McCullough, Melanie Harrison-Dunn, Paul L. Raymer*. To be submitted to Crop Science.

ABSTRACT

Mutations occur naturally in the carboxyl transferase domain of acetyl-coenzyme A carboxylase that result in resistance to ACCase inhibiting herbicides. The development of a weed control system that utilized these mutations as a source of resistance would make the management of hard to control grassy weeds much more efficient. This research surveys 24 species of warm-season, cool-season, and grassy weed species for the presence of mutations at amino acid positions 1781, 1999, 2027, 2041, 2076, 2088, and 2096 in reference to *Alopecurus myosuroides* Huds. AJ310767. Resistance mutations were found at 1781 in three of the 24 species surveyed. Two of these mutations confirm previously reported results in *Festuca rubra* L. and *Poa annua* L. *Festuca ovina* L. has been reported to contain a resistant form of ACCase. Our research now shows this resistance to be due to the presence of the resistant leucine allele at 1781. The results of this research will aid in the development of a herbicide resistance system that could enhance weed control options in many turfgrass species.

Abbreviations: ACCase, Acetyl-coenzyme A carboxylase; APP, aryloxyphenoxypropionate; CHD, cylcohexanedione; PPZ, phenylpyrazoline; Ile, isoleucine; Leu, leucine; Trp, tryptophan; Cys, cysteine; Asn, asparagine; Val, valine; Asp, aspartic acid; Gly, glycine; Arg, arginine; Ala, alanine; CT, carboxyl transferase; KNO_3, potassium nitrate; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

INTRODUCTION

Acetyl-coenzyme A carboxylase (ACCase) (EC.6.4.1.2) is a key enzyme involved in the production of fatty acids that are used in the formation of cell membranes. The homomeric plastidic ACCase is the target for ACCase inhibiting herbicides in the monocotyledon Poaceae. In dicot species, the plastidic ACCase is multimeric and not sensitive to ACCase inhibiting herbicides therefore making ACCase inhibiting herbicides specific to the Poaceae family (Yu et al., 2007). There are three chemical groups of ACCase inhibiting herbicides, aryloxyphenoxypropionate (APP), cylcohexanedione (CHD), and phenylpyrazoline (PPZ). ACCase inhibiting herbicides were introduced in 1978 and are widely used to control grass species. Resistance to ACCase inhibiting herbicides occurs naturally and a total of 42 Poaceae species worldwide have been documented with resistance (Heap, 2012).

There are two main mechanisms of resistance to ACCase inhibitors. Metabolic resistance refers to the plant's ability to metabolize the herbicide to a less toxic or non-toxic form. Metabolic resistance to ACCase inhibiting herbicides is not well understood but normally provides only moderate levels of resistance and has been reported in many species (Preston et al., 1996; Romano et al., 1993; Vila-Aiub et al., 2005). The other type of resistance, known as site of action resistance or target site resistance, is conferred by the presence of single base pair mutations in the CT domain of the ACCase enzyme which is thought to affect the binding of the ACCase inhibiting herbicides (Delye et al., 2005; Devine, 1997; Liu et al., 2007). In total, eight resistance DNA substitutions have

been identified in various Poaceae species at seven amino acid positions (Powles and Yu, 2010), which will henceforth be referred to as mutations sites. Each of these mutations is a single base pair substitution in the gene that encodes chloroplastic ACCase (Devine, 1997). The most commonly found resistance mutation is the 1781 Ile to Leu change in the carboxyl transferase domain of the plastidic ACCase gene, where the codon number is in reference to *Alopecurus myosuroides* Huds. The other mutation sites include 1999 Trp to Cys, 2027 Trp to Cys, 2041 Ile to Asn, 2041 Ile to Val, 2078 Asp to Gly, 2088 Cys to Arg, and 2096 Gly to Ala (Delye et al., 2002).

Recently, resistance to the ACCase inhibiting herbicide sethoxydim was developed using *in vitro* selection to select for a naturally occurring 1781 Ile to Leu mutation in the turfgrass seashore paspalum (*Paspalum vaginatum,* Sw.) (Heckart et al., 2010). This approach could be used to develop herbicide resistant genotypes for other warm-season and cool-season turf species. Once developed, these genotypes could have a significant impact on turfgrass management and make control of hard to control grassy weeds much easier. While these resistance mutations are known and have been documented in many Poaceae species, turfgrass species and their common grassy weeds have not been surveyed for the presence of these mutations. A better understanding of the occurrence of these mutations in turfgrass and weedy grass species is needed to focus the development of ACCase inhibitor resistance weed control systems for maximum benefit.

There are multiple turfgrass species with moderate to high levels of tolerance to ACCase inhibiting herbicides. One or more ACCase inhibiting herbicides are registered for use in several of these species. Envoy Plus (Valent U.S.A. Corporation, Walnut

Creek, CA) contains the active ingredient clethodim and is registered for use in centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) (Anonymous, 2007). Illoxan (Bayer Environmental Science, Research Triangle Park, NC) contains diclofop-methyl and is registered for use to control goosegrass (*Eleusine indica* L.) in bermudagrass (*Cynodon dactylon* [L.] Pers.) on golf courses (Anonymous, 2012). Acclaim Extra (Bayer Environmental Science, Research Triangle Park, NC) has the active ingredient fenoxaprop and is registered for use on Kentucky bluegrass (*Poa pratensis* L.), fine fescues (*Festuca ovina* L. and *Festuca rubra* L.), tall fescue (*Festuca arundinacea* Schreb.), and zoysiagrass (*Zoysia japonica* Steud. and *Zoysia matrella* [L.] Merr.) (Anonymous, 2005). Fusilade II (Syngenta Professional Products, Greensboro, NC) contains the active ingredient fluazifop-butyl and is registered for use in tall fescue (*Festuca arundinacea* Schreb.) and zoysiagrass (*Zoysia japonica* and *Zoysia matrella*) (Anonymous, 2009). Segment (BASF Specialty Products, Research Triangle Park, NC) contains sethoxydim and is registered for use in centipedegrass (*Eremochloa ophiuroides*) and fine fescues (*Festuca ovina* and *Festuca rubra*) (Anonymous, 2010). The source of resistance to ACCase inhibiting herbicides for bermudagrass, centipedegrass, Kentucky bluegrass, tall fescue and zoysiagrass is not known. The high level of resistance in these species could possibly be due to the presence of a resistance conferring mutation at any of the seven amino acid positions known to confer ACCase inhibitor resistance.

The objectives of this research were to: 1) optimize the PCR conditions for primers previously published to amplify the region of DNA sequence that contains the mutations known to confer resistance to ACCase inhibiting herbicides; 2) develop and

test new primers in species or samples where amplification was unsuccessful with previously published primers ; and 3) analyze the sequence data obtained to determine the amino acid sequence at each of the mutation sites; 4) look for presence of new SNPs in resistant species.

MATERIALS AND METHODS

Plant Material and DNA Extraction

Plant material was obtained from the USDA, National Plant Germplasm System (USDA, 2011). In total, 89 accessions representing 24 species were surveyed including eight warm-season turfgrass species, eight cool-season turfgrass species, and eight grassy weed species (Appendix A). In an effort to maximize genetic diversity for each species, accessions chosen within each taxon based on geographic origin. Material was received as either seed or vegetative material (Appendix A). Seeds were germinated in transparent polystyrene boxes (Hoffman Manufacturing, Inc., Albany, OR) with two layers of germination paper (Anchor Paper, St. Paul, MN). Fifteen ml of 0.2% KNO_3 was added, and seeds placed on germination paper. Warm-season and grassy weed species were germinated in a growth chamber at 35°/20° C day/night temperature with an eight-hour photoperiod. Cool-season species were germinated in a growth chamber at 25°/20°C day/night temperature with an eight-hour photoperiod. When seedlings reached 3 cm in height, approximately 0.5 g total of fresh leaf tissue was obtained from 10-15 seedlings for each accession. For the vegetative samples, approximately 0.5 g total of fresh leaf tissue was sampled from 10-15 leaves of each accession. Fresh leaf tissue was place in a screw cap microcentrifuge tube with three ceramic grinding beads and 700 μL CTAB

buffer and pulverized using a Retsch MM300 mixer mill (RETSCH Inc., USA). DNA was extracted according to the CTAB method (Reichardt and Rogers 1997). DNA quality was verified by running an aliquot of each sample in a 1% agarose gel (DNA grade agarose [FischerBiotech]).

Primer Development and PCR Conditions

Two mutation containing regions were amplified - one region containing the 1781 Ile to Leu mutation site and one containing the 1999 Trp to Cys through 2096 Gly to Ala mutation sites. For the 1781 region, the initial primer pair tested was ACCF5/ACCR5 (Delye et al., 2002). If a high quality PCR product was not obtained, an annealing temperature gradient was used, (+/- four degrees) of the annealing temperature published. If a high quality PCR product was obtained for a temperature in the gradient PCR, that temperature was used. For samples with no amplification, the primer set sv384f /sv384r (Heckart et al., 2010) was tested. The process mentioned above using a gradient of annealing temperatures was repeated for samples where a high quality PCR product was not obtained. Lastly, for samples where amplification products were still not obtained, primers were designed by using sequence data already obtained. Sequences of 20-30 base pairs that were highly conserved in the regions 100-200 base pairs before and after the mutation sites were selected in the samples were sequences were already obtained and the melting temperature of each potential primer sequence calculated using the idtdna.com website. Forward and reverse sequences with melting temperatures within one degree were ordered for testing. One primer pair POA2F/POA2R was developed and optimized to amplify the region containing 1781.

The same approach was used to amplify the region containing the 1999-2096 mutation sites starting with the previously published primer set ACCF1/ACCR1 (Yu et al., 2007), followed by primer set ACcp4/ACcp2R (Delye and Michel, 2005), then primer set svac2f/svac1r (Heckart et al., 2010). When previously published primers were unsuccessful the following primers were designed using the aforementioned method - POA3F/WS1R, POA3F/POA3R, and WS2F/WS1R. These novel primer sets were then tested and optimized for genotypes where amplification products could not be obtained using published primer sets.

All primers were obtained from Integrated DNA Technologies (San Diego, CA, USA, http://www.idtdna.com). PCR was performed using 20 nanograms of genomic DNA in a 20 μL total volume with a final concentration of 1X PCR buffer, 0.8 pmol/μL each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 0.05 U/μL *Taq* DNA polymerase (Promega). The conditions for each specific primer pair are listed in Table 2-2 and Table 2-3.

PCR Product Verification,Purification, and Sequencing

An aliquot of each PCR product was run in a 1% agarose gel (DNA grade agarose [FischerBiotech]) to verify a single product of the expected size for the primer pair used. The remaining PCR product was then purified using the DNA Clean & Concentrator 5 kit (Zymo Research Inc., Orange, Calif.). Each purified DNA sample was sequenced both forward and reverse. Briefly, a 15uL volume containing ten ng of purified DNA mixed with 25 pmol of corresponding primer were loaded in plates and shipped per instruction of GENEWIZ Inc. (South Plainfield, NJ). Sequences were analyzed using Geneious v

5.4 software (Drummond et al., 2011). Sequences were aligned and compared to *Alopecurus myosuroides* nucleotide sequence AJ310767 as a reference with all known mutation sites annotated. Sequence data from all accessions were then translated and compared with the translation of *Alopecurus myosuroides* AJ310767 as a reference.

RESULTS

Primer Binding and DNA Amplification

In total there were 89 genotypes where DNA was extracted and available for amplification. Three different primer pairs were used to amplify the region containing the 1781 mutation. Two previously published primer pairs, ACCF5/ACCR5 (Delye et al., 2002), sv384f/sv384r (Heckart et al., 2010), and the newly developed primer POA2F/POA2R. The primer sets used to amplify the 1781 region are shown in Table 2-2 with unsuccessful amplification only occurring in *Z. matrella*. The 1999-2096 region was amplified successfully using six different primer sets including three previously published sets svac2f/svac1r (Heckart et al., 2010), ACCF1/ACCR1 (Yu et al., 2007), and ACcp4/ACcp2R (Delye and Michel, 2005) and three newly developed sets POA3F/WS1R, POA3F/POA3R, and WS2F/WS1R. The primer sets used to amplify the 1999-2096 region are shown in Table 2-3 with unsuccessful amplification occurring in *Bouteloua dactyloides, Digitaria ciliaris, Digitaria sanguinalis, Paspalum dilatum, Paspalum distichum,* and *Paspalum notatum*.

Mutation Site Sequence Results

Sequence data was generated for the region containing the 1781 mutation site in 23 of 24 species surveyed with only *Zoysia matrella* failing to produce sequence data. Twenty of the 23 species contained the wild type susceptible isoleucine amino acid at that position (data shown in Table 2-4). Three species contained a resistance conferring mutation for 1781. All genotypes of *Festuca rubra* and *F. ovina* contained the leucine codon, CTA or TTA, at position 1781. For *Poa annua,* genotypes PI 659870 and W6 28171 were homozygous for the TTA codon at 1781 while genotypes PI 442543 and PI 236900 were heterozygous due to the presence of both T and A nucleotides at the first position of the 1781 codon. Figure 2-1 shows examples of each type of mutation chromatogram.

The region containing mutation sites 1999, 2027, 2041, 2076, 2088, and 2096 had six species where amplification of that region was unsuccessful (*B. dactyloides, D. ciliaris, D. sanguinalis, P. dilatum, P. distichum, and P. notatum*). For these species, DNA amplification was not obtained using any of the primer pairs tested. Further research is needed to develop primers that amplify this mutation-containing region in these species. The 1999-2096 mutation-containing region of the remaining 18 species (*Agrostis capillaris, Agrostis stolonifera, Cynodon dactylon, Cynodon hybrid, Cynodon transvaalensis, Eleusine indica, Eremochloa ophiuroides, Festuca arundinacea, Festuca ovina, Festuca rubra, Lolium multiflorum, Lolium perenne, Poa annua, Poa pratensis, Paspalum vaginatum, Stenotaphrum secundatum, Zoysia japonica,* and *Zoysia matrella*) was successfully amplified using various primers and conditions (data shown in Table 2-

3). These species all contained wild type susceptible alleles for the mutation sites 1999-Trp, 2027-Trp, 2041-Ile, 2076-Asp, 2088-Cys, and 2096-Gly (Table 2-4). We therefore conclude that any observed resistance to ACCase inhibiting herbicides in these species is not due to the presence of any of previously reported mutations in the 1999-2096 region.

Sequence data from these two regions were analyzed to determine if any of the SNPs present could possibly be the source for the observed resistance for species with ACCase inhibiting herbicides registered for use. One SNP was found only in *Cynodon* species at amino acid position 1755. The amino acid glutamine was present at this location for *Cynodon* species. Diclofop-methyl is registered for use in *Cynodon* species and this SNP could be the source for resistance for diclofop in Cynodon, but further research is needed to confirm this SNP as the source of resistance.

DISCUSSION

Mutation Site Sequence

The homozygous leucine allele at 1781 for *Festuca rubra* confirms the results previously published by Delye and Michel (2005). Having only leucine present at 1781 for *Festuca rubra* and *F. ovina* suggests that this is the only allele present in those species and that the resistant allele is the wild type in these species. Delye and Michel (2005) also sequenced *Poa annua* and found both isoleucine and leucine alleles. Our results confirmed their findings. We found two genotypes that contained only leucine at position 1781 and two genotypes that contained both isoleucine and leucine at position 1781. This suggests that some genotypes are fixed for this mutation while others are still

segregating and implies that some genotypes may be homozygous for the susceptible allele.

Tall fescue was surveyed in a previous study that found it did not contain mutations at five of the seven mutation sites (1781, 2027, 2041, 2076, 2096) (Delye and Michel, 2005). Our results now confirm that tall fescue also does not contain the 1999-Trp or the 2088-Cys mutations. *Cynodon dactylon, Eremochloa ophiuroides, Poa pratensis*, *Zoysia japonica*, and *Z. matrella* all have ACCase inhibiting herbicides registered for use on them and are known to have resistance to various ACCase inhibiting herbicides. Our research determined that the source of this resistance is not due to the presence of resistance conferring mutations at any of the seven known mutation sites for the genotypes tested excluding *Z. matrella* at 1781, which sequence data was not generated. There was one SNP observed at amino acid position 1755 only observed in *Cynodon* species that may be the source of resistance to diclofop-methyl in bermudagrass. Further research is needed to confirm that this mutation is the source of the resistance. All other SNPs did not associate with resistant species.

The results of this research will aid in the development and design of herbicide resistance weed control systems for turfgrass. For species that do not contain resistance mutations, ACCase inhibitor resistance could be developed using the *in vitro* method utilized to develop sethoxydim resistant seashore paspalum (Heckart et al., 2010). Once developed, the resistance could be used to manage grassy weeds that are ACCase inhibitor susceptible. Further research is needed to better understand the nature of resistance observed in species that do not have known resistance mutations.

LITERAURE CITED

Anonymous. 2005. Acclaim Extra herbicide label, Bayer Environmental Science, Research Triangle Park, NC.

Anonymous. 2007. Envoy Plus herbicide label, Valent U.S.A. Corporation, Walnut Creek, CA.

Anonymous. 2009. Fusilade II herbicide label, Syngenta Crop Protection, Greensboro, NC.

Anonymous. 2010. Segment herbicide label, BASF Corporation, Research Triangle Park, NC.

Anonymous. 2012. Illoxan herbicide label, Bayer Environmental Science, Research Triangle Park, NC.

Delye C. and Michel S. 2005. 'Universal' primers for PCR-sequencing of grass chloroplastic acetyl-CoA carboxylase domains involved in resistance to herbicides. Weed Res. 45:323-330.

Delye C., Matejicek A., and Gasquez J. 2002. PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manage Sci. 58:474-478.

Delye C., Zhang X.Q., Michel S., Matejicek A., and Powles S.B. 2005. Molecular bases for sensitivity to acetyl-coenzyme a carboxylase inhibitors in black-grass. Plant Physiol. 137:794-806.

Devine M.D. 1997. Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibitors: a review. Pestic. Sci. 51:259-264.

Drummond A.J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir R., Stones-Havas S., Sturrock S., Thierer T., and Wilson A. 2011. Geneious v5.4, Available from http://www.geneious.com/

Heap I.M. 2012. International survey of herbicide-resistant weeds. Weed Science. http://www.weedscience.org (March 21, 2012)

Heckart D.L., Parrott W.A., and Raymer P.L. 2010. Obtaining sethoxydim resistance in seashore paspalum. Crop Sci. 50:2632-2640.

Liu W.J., Harrison D.K., Chalupska D., Gornicki P., O'Donnell C.C., Adkins S.W., Haselkorn R., and Williams R.R. 2007. Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. Proc. Natl. Acad. Sci. USA. 104:3627-3632.

Powles S.B. and Yu Q. 2010. Evolution in action: plants resistant to herbicides. in: S. Merchant, et al. (Eds.), Annu. Rev. of Plant Biol. 61:317-347.

Preston C., Tardif F.J., Christopher J.T., and Powles S.B. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochem. Physiol. 54:123-134.

Reichardt M. and Rogers S. 1997. Preparation of genomic DNA from plant tissue. In: Ausubel F.M. et al. (eds), Current Protocols Molecular Biology. John Wiley & Sons, Inc., New York, pp. 2.3.3–2.3.7.

Romano M.L., Stephenson G.R., Tal A., and Hall J.C. 1993. The effect of monooxygenase and glutathione-S-tranferase inhibitors on the metabolism of

diclofop-methyl and fenoxaprop-ethyl in barley and wheat. Pesticide Biochem. Physiol. 46:181-189.

USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1371750 (6 February 2011)

Vila-Aiub M.M., Neve P., and Powles S.B. 2005. Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. New Phytol. 167:787-796.

Yu Q., Collavo A., Zheng M.Q., Owen M., Sattin M., and Powles S.B. 2007 Diversity of acetyl-coenzyme a carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. Plant Physiol. 145:547-558.

Table 2-1. Base pair sequences of all primers used to sequence the ACCase inhibitor resistance mutation containing regions of the ACCase enzyme**.**

Forward		Reverse		
1781 Region				
Name	Sequence	Name	Sequence	Reference
ACCF5	AATGGGTCGTGGGGCACTCCTATAATTCC	ACCR5	GCTGAGCCACCTCAATATATTAGAAACACC	Delye et al. 2002
sv384f	CGGGGTTTCAGTACATTTAT	sv384r	GATCTTAGGACCACCCAACTG	Heckart et al. 2010
POA2F	AGTGGCGAAATTAGGTGG	POA2R	CTGCACGAGGATCACATG	
1999-2096 Region				
Name	Sequence	Name	Sequence	Reference
ACCF1	CACAGACCATGATGCAGCTC	ACCR1	CTCCCTGGAGTTGTGCTTTC	Yu et al. 2007
ACcp4	CAGCITGATTCCCAIGAGCGITC	ACcp2R	CCATGCAITCTTIGAGITCCTCTGA	Delye and Michel 2005
svac2f	AATTCCTGTTGGTGTCATAGCTGTGGAG	svac1r	AATTCCTGTTGGTGTCATAGCTGTGGAG	Heckart et al. 2010
POA3F	CATGTGATCCTCGTGCAG	WS1R	AGAACCTCCAGGAGACTAG	
POA3F	CATGTGATCCTCGTGCAG	POA3R	CTCAATCAACCCTTGAGG	
WS2F	GTGTTGATGACGGTCAAGG	WS1R	GATAGACCAGATACGCACC	

Table 2-2. Summary of primer sets and PCR conditions used for amplification of the 1781 mutation-containing region of ACCase in 24 grass species.

			1781	
Type/Species	**Reads**	**Forward**	**Reverse**	**PCR Conditions**
Cool-season turfgrass				
Agrostis capillaris	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Agrostis stolonifera	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Festuca arundinacea	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Festuca ovina	8	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Festuca rubra	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Lolium multiflorum	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Lolium perenne	8	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Poa pratensis	2	POA2F	POA2R	5’ @ 95°C, 35x(30” @ 95°C, 30” @ 60 °C, 30” @ 72°C), 7’ @ 72°C
Warm-season turfgrass				
Bouteloua dactyloides	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Cynodon hybrid	2	sv384F	sv384R	4’ @ 95°C, 35x(30” @ 95°C, 30” @ 52.7 °C, 30” @ 72°C), 7’ @ 72°C
	1	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Cynodon transvaalensis	8	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Eremochloa ophiuroides	8	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
	2	sv384F	sv384R	4’ @ 95°C, 35x(30” @ 95°C, 30” @ 52.7 °C, 30” @ 72°C), 7’ @ 72°C
Paspalum vaginatum	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
	7	sv384F	sv384R	4’ @ 95°C, 35x(30” @ 95°C, 30” @ 52.7 °C, 30” @ 72°C), 7’ @ 72°C
Stenotaphrum secundatum	8	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Zoysia japonica	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Zoysia matrella	-†	-	-	-
Grassy weed				
Cynodon dactylon	10	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
	2	sv384F	sv384R	4’ @ 95°C, 35x(30” @ 95°C, 30” @ 52.7 °C, 30” @ 72°C), 7’ @ 72°C
Digitaria ciliaris	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Digitaria sanguinalis	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Eleusine indica	6	POA2F	POA2R	5’ @ 95°C, 35x(30” @ 95°C, 30” @ 60 °C, 30” @ 72°C), 7’ @ 72°C
	-	-	-	-
Paspalum dilatum	6	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Paspalum distichum	4	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
	2	sv384F	sv384R	4’ @ 95°C, 35x(30” @ 95°C, 30” @ 52.7 °C, 30” @ 72°C), 7’ @ 72°C
Paspalum notatum	6	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
Poa annua	2	ACCF5	ACCR5	4’ @ 94°C, 35x(30” @ 95°C, 30” @ 64 °C, 30” @ 72°C), 7’ @ 72°C
	5	POA2F	POA2R	5’ @ 95°C, 35x(30” @ 95°C, 30” @ 60 °C, 30” @ 72°C), 7’ @ 72°C

† **-** Dash indicates unsuccessful PCR amplification. No sequence data generated.

Table 2-3. Summary of primer sets and PCR conditions used for amplification of the 1999-2096 mutation-containing region of ACCase in 24 grass species.

			1999-2096	
Type/Species	**Reads**	**Forward**	**Reverse**	**PCR Conditions**
Cool-season turfgrass				
Agrostis capillaris	10	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Agrostis stolonifera	10	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Festuca arundinacea	10	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Festuca ovina	8	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Festuca rubra	8	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Lolium multiflorum	8	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Lolium perenne	8	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Poa pratensis	2	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Warm-season turfgrass				
Bouteloua dactyloides	-†	-	-	-
Cynodon hybrid	2	POA3F	WS1R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 54 °C, 30" @ 72°C), 7' @ 72°C
Cynodon transvaalensis	10	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Eremochloa ophiuroides	2	ACcp4F	ACcp2R	30" @ 95°C, 37x(10" @ 95°C, 15" @ 60 °C, 45" @ 72°C), 10' @ 72°C
	2	WS2F	WS1R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 54 °C, 30" @ 72°C), 7' @ 72°C
Paspalum vaginatum	6	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
	2	SVAC2F	SVAC1R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 58 °C, 30" @ 72°C), 7' @ 72°C
Stenotaphrum secundatum	4	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
	4	WS2F	WS1R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 54 °C, 30" @ 72°C), 7' @ 72°C
Zoysia japonica	4	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
	2	POA3F	POA3R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 60 °C, 30" @ 72°C), 7' @ 72°C
Zoysia matrella	4	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Grassy weed				
Cynodon dactylon	6	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
	2	WS2F	WS1R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 54 °C, 30" @ 72°C), 7' @ 72°C
Digitaria ciliaris	-	-	-	-
Digitaria sanguinalis	-	-	-	-
Eleusine indica	4	POA3F	POA3R	5' @ 95°C, 35x(30" @ 95°C, 30" @ 60 °C, 30" @ 72°C), 7' @ 72°C
	2	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C
Paspalum dilatum	-	-	-	-
Paspalum distichum	-	-	-	-
Paspalum notatum	-	-	-	-
Poa annua	8	ACCF1	ACCR1	4' @ 94°C, 35x(30" @ 94°C, 30" @ 62 °C, 30" @ 72°C), 7' @ 72°C

† - Dash indicates unsuccessful PCR amplification. No sequence data generated.

Table 2-4. Amino acid translation at each of the seven mutation sites for the 24 grass species where DNA sequences were obtained of the two regions in the CT domain of the acetyl coenzyme A carboxylase enzyme.

	Genotypes	1781†	1999	2027	2041	2076	2088	2096
Wild Type Susceptible		**Ile**	**Trp**	**Trp**	**Ile**	**Asp**	**Cys**	**Gly**
Cool-season species								
Agrostis capillaris	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Agrostis stolonifera	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Festuca arundinacea	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Festuca ovina	5	Leu	Trp	Trp	Ile	Asp	Cys	Gly
Festuca rubra	5	Leu	Trp	Trp	Ile	Asp	Cys	Gly
Lolium multiflorum	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Lolium perenne	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Poa pratensis	1	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Warm-season species								
Bouteloua dactyloides	1	Ile	-‡	-	-	-	-	-
Cynodon hybrid	2	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Cynodon transvaalensis	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Eremochloa ophiuroides	4	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Paspalum vaginatum	6	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Stenotaphrum secundatum	4	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Zoysia japonica	3	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Zoysia matrella	2	-	Trp	Trp	Ile	Asp	Cys	Gly
Grassy weed species								
Cynodon dactylon	6	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Digitaria ciliaris	1	Ile	-	-	-	-	-	-
Digitaria sanguinalis	1	Ile	-	-	-	-	-	-
Eleusine indica	5	Ile	Trp	Trp	Ile	Asp	Cys	Gly
Paspalum dilatum	3	Ile	-	-	-	-	-	-

Paspalum distichum	3	Ile	-	-	-	-	-	-
Paspalum notatum	**3**	**Ile**	**-**	**-**	**-**	**-**	**-**	**-**
Poa annua	**4**	**Ile/Leu Leu**	**Trp**	**Trp**	**Ile**	**Asp**	**Cys**	**Gly**

† - ACCase codon number in reference to *Alopecurus myosuroides* Genbank AJ310767

‡ - Dashes indicate no data due to unsuccessful PCR amplification

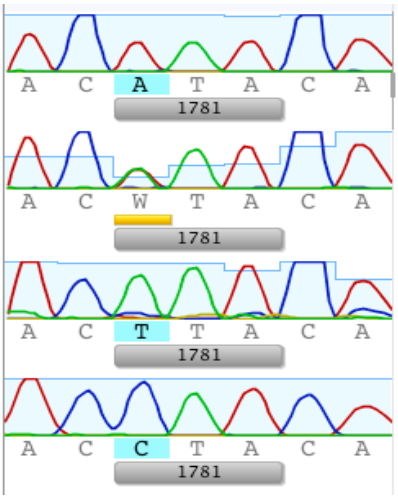
(ATA) Isoleucine Susceptible wild type	
(ATA/TTA) Isoleucine/Leucine Resistant	
(TTA) Leucine Resistant	
(CTA) Leucine Resistant	

Figure 2-1. Sequence chromatograms of possible mutations for mutation site 1781.

CHAPTER 3

RESPONSE OF TURFGRASS AND GRASSY WEEDS TO FENOXAPROP

---

1 Trent M. Tate, Patrick E. McCullough, Melanie Harrison-Dunn, Paul L. Raymer*. To be submitted to Crop Science.

ABSTRACT

The development of a herbicide resistance weed control system for turfgrass using ACCase inhibiting herbicides would provide a means of controlling many problematic grassy weeds. Before this can be developed and implemented, a better understanding is needed of the physiological response for each species to ACCase inhibitors. This study included 80 genotypes of 24 of warm-season, cool-season, and grassy weed species. Three rates, 0, 400, and 1200 g a.i. ha^{-1} of the ACCase inhibiting herbicide, fenoxaprop were sprayed at 87 L ha^{-1} and the resulting plant injury evaluated. Species injury means were separated using Fisher's protected LSD at α 0.05. Species in the statistical group with the lowest injury to 400 g a.i. ha^{-1} fenoxaprop 30 DAT were categorized as resistant while all others were categorized as susceptible. Resistant species included *Agrostis capillaris, Festuca ovina, F. rubra, Lolium multiflorum, L. perenne, Paspalum dilatatum, Poa annua, Zoysia japonica,* and *Z. matrella* with all others species tested susceptible.

Abbreviations: ACCase, Acetyl-coenzyme A carboxylase; APP, aryloxyphenoxypropionate; CHD, cylcohexanedione; PPZ, phenylpyrazoline; CT, carboxyl transferase; KNO_3, potassium nitrate; MSO, methylated seed oil; Ile, isoleucine; Leu, leucine; Trp, tryptophan; Cys, cysteine; Asn, asparagine; Val, valine; Asp, aspartic acid; Gly, glycine; Arg, arginine; Ala, alanine

INTRODUCTION

Turfgrass is utilized for as lawns, sports turf, and golf courses. In Georgia, it is estimated that turfgrass encompasses over 1.6 million acres (Waltz et al., 2010). Many species of Poaceae are used as turfgrass due to their desirable characteristics. One aspect that is common to all species and cultivars in highly managed situations is weed control.

Turfgrass has many types of weeds that can be present, but the most difficult to manage are perennial grassy weeds. Currently there are very few options for the removal of perennial grasses from turfgrass, for example, the selective removal of common bermudagrass (*Cynodon dactylon* (L.) Pers.) from warm-season turfgrass species, such as seashore paspalum (*Paspalum vaginatum* Sw.) or bermudagrass (*Cynodon hybrid*) is difficult using currently available herbicides. Control of many problematic grassy weeds would be much more effective if herbicide resistant turfgrass cultivars were developed similar to the glyphosate resistant cultivars used in agricultural crops. In these systems, herbicide resistance is introduced into the crop thereby providing enhanced selectivity and the ability to safely control problematic weeds.

Acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicides are a class of herbicides that control weedy grasses. Acetyl-coenzyme A carboxylase (EC.6.4.1.2) is the enzyme that catalyzes the metabolic reaction in the formation of fatty acids from the carboxylation of acetyl-CoA to malonyl-CoA. ACCase can exist in plants as two distinct forms. The heteromeric prokaryotic ACCase is composed of multiple subunits, while the homomeric eukaryotic ACCase is a large multi-domain protein (Harwood, 1988). Plants

contain both plastidic and cytosolic forms of ACCase. In grasses, the plastidic ACCase is homomeric, whereas in most dicots the plastidic form of ACCase is mutlimeric (Sasaki et al., 1995). This homomeric plastidic ACCase present in grasses is the target site for ACCase inhibiting herbicides thus making them grass specific in most cases and effective in controlling grasses (Herbert et al., 1997). ACCase inhibiting herbicides bind irreversibly to ACCase inhibiting the catalytic function of the enzyme and disrupting fatty acid synthesis (Burton et al., 1991).

There are three chemical groups within the ACCase inhibiting herbicides, the aryloxyphenoxypropionates, the cyclohexanediones, and the phenylpyrazolines (Powles and Yu, 2010). Since their introduction in 1978 and subsequent widespread use to control various grass weeds, resistance to ACCase inhibiting herbicides has been reported throughout the world. In total, 42 species have developed populations with resistance to various ACCase inhibiting herbicides (Heap, 2012).

Resistance to ACCase inhibitors generally falls into two major categories, herbicide metabolism (Preston et al., 1996; Romano et al., 1993; Vila-Aiub et al., 2005), and altered site of action that reduces the enzyme to herbicide binding affinity (Delye et al., 2005; Devine and Shukla, 2000; Liu et al., 2007). The level of resistance conferred varies with the type of resistance, with site of action mutations typically conferring the highest levels of resistance. Site of action resistance for ACCase inhibiting herbicides are naturally occurring mutations in the carboxyl transferase domain of the ACCase enzyme. There are seven sites at which mutations have been documented to confer differing levels of resistance to the various ACCase inhibiting herbicides. Each of the seven mutation sites confers resistance due to a nucleotide substitution that changes the amino acid

transcribed for that position and eight mutations have been documented (Powles and Yu, 2010).

A method was recently developed to utilize one of these naturally occurring mutations to develop ACCase inhibitor resistant turfgrass cultivars (Heckart et al., 2010). The success achieved using this technique brings the possibility of the development of an ACCase inhibitor resistant weed control system for turfgrass. However, a better understanding of the resistance spectrum of both warm- and cool-season turfgrass species, as well as their common grassy weeds is needed to prioritize development of herbicide resistance systems for turfgrass.

Fenoxaprop is an aryloxyphenoxypropionate herbicide that inhibits ACCase and marketed under the U.S. trade name Acclaim Extra (Bayer Environmental Science, Montville, NJ). It has been shown that resistance to fenoxaprop is conferred if any one of six of the ACCase mutations is present (1781 Ile to Leu, 2027 Trp to Cys, 2041 Ile to Asn, 2041 Ile to Val, 2078 Asp to Gly, 2096 Gly to Ala) (Delye, 2005). Based on these reports, it appears that any of the known mutations are likely to confer resistance to fenoxaprop, whereas resistances to other herbicides are conferred by only a few specific mutations. For this reason, we chose fenoxaprop as the ACCase inhibiting herbicide for screening plant materials since the presence of any of the known mutations should result in resistance to fenoxaprop.

The objective of this research was to determine the response of multiple accessions of 24 grass species to applications of the ACCase inhibiting herbicide fenoxaprop.

MATERIALS AND METHODS

Plant Material

Plant material was obtained from the USDA, National Plant Germplasm System (USDA, ARS, National Genetic Resources Program, 2011) as either seed or vegetative clones. There were a total of 24 species included in this evaluation with one to four genotypes per species depending on availability of plant material for a total of 80 genotypes tested. Vegetative material was collected, planted in 10 cm pots, and maintained at 5-8 cm height with sheers. Grasses were fertilized every two weeks with a water soluble N-P-K (28-7-14) fertilizer (LESCO MacroN, Cleveland, OH) for five weeks prior to application of herbicides treatments. For genotypes where seed was the source of plant material (see Appendix A), seeds were germinated in a 0.2 % KNO_3 solution and seedlings were transplanted after 10 days into individual 2.5 cm cells of a 72 cell greenhouse flat. Each species had three flats total (one for each spray treatment 0, 400, and 1200 g a.i. ha^{-1}). Each flat contained between one and four accessions. For each accession there were between two and 18 individuals. Seedlings were allowed to grow to a height of approximately 10-15 cm and fertilized every two weeks with aforementioned fertilizer prior to application of fenoxaprop.

Fenoxaprop Screen

Fenoxaprop treatments used in this experiment were 0, 400, and 1200 g a.i. ha^{-1} applied with 1% MSO. Plant material was limited and therefore genotypes were used as replicates for classification of herbicide response within a species. In the case of clonally

propagated species, a single plant of each genotype was sprayed with each of the three rates of fenoxaprop. Experimental factors were herbicide rates and replications (genotypes) arranged in a completely randomized design. For seeded species, genotypes were again used as replication with each species and experimental units consisted of two to 18 individual plants of each genotype arranged in a completely randomized design.

Treatments were applied using a spray chamber calibrated to deliver a rate of 87 liters per hectare. Injury ratings were taken at 0, 18, and 30 days after treatment (DAT). Percent injury was rated 0-100 for each genotype, where 0 was no visible injury and 100 was complete plant death. Species injury means were separated using Fisher's protected LSD at α 0.05. Species in the top statistical group at 400 g a.i. ha^{-1} fenoxaprop rate at 30 DAT rating were categorized as resistant while all others categorized as susceptible.

No further screening was done if all genotypes within a species showed a similar herbicide response. However, further screening was carried out when genotypic differences were observed within a species. In these cases, a second stage herbicide test was carried out using additional rates of fenoxaprop and three replications to confirm our initial results.

Additional Dose Response for Species with Genotypic Variation in Fenoxaprop Response

Second stage testing for genotypes with genotypic variation were obtained by separating tillers of the individual seedlings from the 0 g a.i. ha^{-1} treatment from the initial herbicide screen for 'PI 203728', 'PI 234892', and 'PI 221906'. Individual tillers were planted into 3.175 cm diameter potting tubes. All plants were fertilized post planting using aforementioned fertilizer, allowed to grow for three weeks, and fertilized

again three days prior to spraying. 'PI 364981' seashore paspalum, 'Mauna Kea' seashore paspalum, and 'Greystone' tall fescue were obtained using vegetative cuttings taken from previously untreated plants and transplanting them into 3.175 cm diameter potting tubes.

Fenoxaprop rates used were 0, 400, 800, 1200, and 1600 g a.i. ha^{-1} applied with 1% MSO. The experimental design was a randomized complete block with three replications. Each treatment was sprayed in a spray chamber at 87 L ha^{-1} as previously described. Visual ratings of percent plant injury were taken at 21 and 28 DAT using the scale previously described.

RESULTS

Fenoxaprop Screen

Results of the fenoxaprop dose response experiments conducted on 24 grass species are presented in Table 3-1. Of the 24 species included in the screen, nine species, colonial bentgrass (*Agrostis capillaris* L.), sheeps fescue (*Festuca ovina* L.), red fescue (*Festuca rubra* L.), annual ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), dallisgrass (*Paspalum dilatatum* Poir.), annual bluegrass (*Poa annua* L.), and zoysiagrass (*Zoysia japonica* Steud. and *Z. matrella* [L.] Merr.) were categorized as resistant due to being in the statistical group with the lowest injury. Three species, creeping bentgrass (*Agrostis stolonifera* L.), tall fescue (*Festuca arundinacea* Schreb.), and seashore paspalum, had one, two, and one accession, respectively, that showed an injury response different than the other accessions within those particular species. The susceptible species (species not in top statistical group for injury) included

Kentucky bluegrass (*Poa pratensis* L.), tall fescue, goosegrass (*Eleusine indica* [L.] Gaertn.), Texas bluegrass (*Poa arachnifera* Torr.), buffalograss (*Bouteloua dactyloides* Nutt.), common bermudagrass, creeping bentgrass, St. Augustinegrass (*Stenotaphrum secundatum* [Watler] Kuntze), African bermudagrass (*Cynodon transvaalensis* Burtt Davy), bahiagrass (*Paspalum distichum* L.), knotgrass (*Paspalum notatum* Flugge), centipedegrass (*Eremochloa ophriuroides* [Munro] Hack.), southern crabgrass (*Digitaria ciliaris* [Retz.] Koeler) and hybrid bermudagrass.

Species with DifferentialResponse

The *Paspalum vaginatum* accession PI364981 in the initial tests showed no injury when fenoxaprop was applied at 400 g a.i. ha^{-1} and 5% injury at 1200 g a.i. ha^{-1} while the other three accessions had between 50 and 98 % injury for both rates at 30 DAT (data not shown). The *Agrostis stolonifera* accession PI221906 had 10 and 20 % injury at 400 and 1200 g a.i. ha^{-1} fenoxaprop respectively, when all other accessions in that species had 100 % injury for both rates at 30 DAT (data not shown). Accessions of *Festuca arundinacea* varied in response to fenoxaprop applications. Accessions PI203728 and PI234892 showed less than 20 % injury both rates while the other accessions PI655116 and PI598923 had 50 to 60 % injury (data not shown).

Additional Dose Response for Species with Genotypic Variation in Fenoxaprop Response

Results of the second stage testing for herbicide response are shown in Table 3-3. The *Festuca arundinacea* genotypes, Greystone (standard cultivar), PI 203728, and PI 234892 showed a response similar to that observed in the initial dose response

experiment indicating that these genotypes do have tolerance to fenoxaprop. Based on these results it is apparent that genotypes of tall fescue vary in tolerance to fenoxaprop.

The repeated fenoxaprop dose response experiments for *Paspalum vaginatum* PI 364981 were different from the first screen. The percent injury mean at 28 DAT was 38% for the 400 g a.i. ha^{-1} treatment level compared to 0% injury at the same rate in the previous test. At the 1200 g a.i. ha^{-1} where initial injury was 0% the repeated dose response injury was 76%. Based on these results, PI 364981 was re-classified as susceptible although it does appear to have better tolerance to fenoxaprop at 400 g a.i. ha^{-1} than Mauna Kea.

The repeated herbicide response of *Agrostis stolonifera* accession PI 221906 at 28 DAT showed 78% for the 400 g a.i. ha^{-1} compared to 10% injury at the same rate in the previous test. At the 1200 rate the repeated herbicide response showed 76% injury compared to 20% injury at that rate in the initial dose response experiment. Based on these results PI 221906 was classified susceptible.

DISCUSSION

The 24 grass species varied greatly in their response to fenoxaprop and genotypic differences were observed among *F. arundinacea* genotypes. *A. capillaris, F. ovina, F. rubra, L. multiflorum, L. perenne, P. dilatatum, P. annua, Z. japonica,* and *Z. matrella* are resistant to fenoxaprop. The resistance found in *Festuca ovina, Festuca rubra,* and *Poa annua* can be explained by the presence of a point mutation at amino acid position 1781 in the carboxyl transferase region of ACCase (Chapter 2). The nature of resistance

or tolerance levels to fenoxaprop for the remaining species, *A. capillaris, L. multiflorum, Z. japonica* and *Z. matrella,* is not known.

Table 3-3 was developed to summarize the potential of fenoxaprop herbicide to control each of the 24 grass species in turfgrass cultivars with (+M) and without (WT) the site of action mutations conferring resistance to fenoxaprop. Our results indicate that the development of cultivars containing site of action mutations conferring resistance to fenoxaprop would be of little to no benefit in colonial bentgrass, sheep fescue, red fescue, annual ryegrass, perennial ryegrass, dallisgrass, annual bluegrass, and zoysiagrass because these species already demonstrate high levels of resistance to fenoxaprop. Turfgrass species that could possibly benefit from this approach to weed control include creeping bentgrass, Texas bluegrass, buffalograss, bermudagrass, centipedegrass, seashore paspalum, and St. Augustinegrass. The development of herbicide resistant cultivars in these species would improve control of common bermudagrass, goosegrass, knotgrass, southern crabgrass, and other problematic weeds in warm-season turf species. Additionally, control of other fenoxaprop susceptible turf species would become possible.

Data from this research provides a better understanding of the response of many cool- and warm-season turfgrass species to fenoxaprop. This information could be useful in establishing priorities of species that would benefit from resistance conferring mutations for an improved weed control system. Such systems could make the management of hard to control grassy weeds more efficient. It will also improve the conversion from one turf species to another by using differential tolerance levels to ACCase inhibitors. This could reduce costs involved with converting less efficient cultivars to improved varieties developed to use fewer inputs such as water and nutrients.

This is vital developing sustainable turfgrass for golf courses, home lawns and other uses. Future research should characterize these species for their response to application of other ACCase inhibiting herbicides and evaluate where this technology could be implemented.

LITERAURE CITED

Burton J.D., Gronwald J.W., Keith R.A., Somers D.A., Gengenbach B.G., and Wyse D.L. 1991. Kinetics of inhibition of acetyl-coenzyme-A carboxylase by sethoxydim and haloxyfop. Pestic. Biochem. Physiol. 39:100-109.

Delye C. 2005. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci. 53:728-746.

Delye C., Zhang X.Q., Michel S., Matejicek A., and Powles S.B. 2005. Molecular bases for sensitivity to acetyl-coenzyme a carboxylase inhibitors in black-grass. Plant Physiol. 137:794-806.

Devine M.D. and Shukla A. 2000. Altered target sites as a mechanism of herbicide resistance. Crop Prot. 19:881-889.

Harwood J.L. 1988. Fatty-acid metabolism. Annu. Rev. of Plant Physiol. and Plant Mol. Biol. 39:101-138.

Heap I.M. 2012. International survey of herbicide-resistant weeds. Weed Science. http://www.weedscience.org (March 21, 2012)

Heckart D.L., Parrott W.A., and Raymer P.L. 2010. Obtaining sethoxydim resistance in seashore paspalum. Crop Sci. 50:2632-2640.

Herbert D., Walker K.A., Price L.J., Cole D.J., Pallett K.E., Ridley S.M., and Harwood J.L. 1997. Acetyl-CoA carboxylase - a graminicide target site. Pestic. Sci. 50:67-71.

Liu W.J., Harrison D.K., Chalupska D., Gornicki P., O'Donnell C.C., Adkins S.W., Haselkorn R., and Williams R.R. 2007. Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. Proc. Natl. Acad. Sci. USA. 104:3627-3632.

Powles S.B. and Yu Q. 2010. Evolution in action: plants resistant to herbicides. in: S. Merchant, et al. (Eds.), Annu. Rev. of Plant Biol. 61:317-347.

Preston C., Tardif F.J., Christopher J.T., and Powles S.B. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochem. Physiol. 54:123-134.

Romano M.L., Stephenson G.R., Tal A., and Hall J.C. 1993. The effect of monooxygenase and glutathione-S-tranferase inhibitors on the metabolism of diclofop-methyl and fenoxaprop-ethyl in barley and wheat. Pesticide Biochem. Physiol. 46:181-189.

Sasaki Y., Konishi T., and Nagano Y. 1995. The compartmentation of acetyl-coenzyme-A carboxylase in plants. Plant Physiol. 108:445-449.

USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1371750 (6 February 2011)

Vila-Aiub M.M., Neve P., and Powles S.B. 2005. Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. New Phytol. 167:787-796.

Waltz C., Murphy T., and Landry G. 2010 Turfgrass industry facts in Georgia. in: U. o. Georgia (Ed.), 2010 Turfgrass Pest Control.

Table 3-1. Percent injury of 24 warm-season, cool-season, and grassy weed species in response to rates of fenoxaprop.

		Fenoxaprop Rate					
		g a.i. ha^{-1}					
		0		400		1200	
Common Name	**Species**	n	Injury	n	Injury	n	Injury
			%		%		%
Annual bluegrass	*Poa annua*	4	0a†	3	0a	4	0a
Sheep fescue	*Festuca ovina*	4	0a	4	0a	4	0a
Red fescue	*Festuca rubra*	4	0a	4	0a	4	0a
Zoysiagrass	*Zoysia japonica*	3	0a	3	2a	3	2ab
Zoysiagrass	*Zoysia matrella*	2	0a	2	0a	1	0‡
Colonial bentgrass	*Agrostis capillaris*	4	0a	4	0a	4	5ab
Annual ryegrass	*Lolium multiflorum*	4	0a	4	0a	4	8ab
Perennial ryegrass	*Lolium perenne*	4	0a	4	0a	4	30bc
Dallisgrass	*Paspalum dilatatum*	2	0a	2	6ab	2	23abc
Kentucky bluegrass	*Poa pratensis*	4	0a	4	23bc	4	68df
Tall fescue	*Festuca arundinacea*	4	0a	4	33bc	4	28bc
Goosegrass	*Eleusine indica*	3	0a	3	40cd	3	75defgh
Texas bluegrass	*Poa arachnifera*	4	0a	4	41cd	4	60d
Buffalograss	*Bouteloua dactyloides*	1	0‡	1	45‡	1	55‡
Seashore paspalum	*Paspalum vaginatum*	4	0a	4	58de	4	72defg
Bermudagrass	*Cynodon dactylon*	3	0a	4	76ef	3	88defgh
Creeping bentgrass	*Agrostis stolonifera*	4	0a	4	78ef	4	80defgh
St. Augustinegrass	*Stenotaphrum secundatum*	4	0a	4	92f	4	91efgh
African bermudagrass	*Cynodon transvaalensis*	4	0a	4	92f	4	96egh
Knotgrass	*Paspalum distichum*	4	0a	4	98f	3	100gh
Bahiagrass	*Paspalum notatum*	3	0a	3	98f	3	100gh

Centipedegrass	*Eremochloa ophiuroides*	4	0a	4	98f	4	100h
Southern crabgrass	*Digitaria ciliaris*	1	0‡	1	100‡	1	100‡
Hybrid bermudagrass	*Cynodon hybrid*	1	0‡	1	100‡	1	98‡

† Species means within rates followed by the same letter are not statistically different according to Fisher's protected LSD at α 0.05.

‡ Value from single observation therefore statistical analysis not performed.

Table 3-2. Mean herbicide injury of tall fescue, seashore paspalum, and creeping bentgrass 28 days after treatment with rates of fenoxaprop herbicide.

		Fenoxaprop Rate (g a.i. ha^{-1})				
		g a.i. ha^{-1}				
		0	400	800	1200	1600
Common Name	**Species/Genotype**	Injury				
Tall Fescue	*Festuca arundinacea*	%				
	Greystone	0a†	8a	8a	3a	3a
	PI 203728	2a	0b	0a	13a	0a
	PI 234892	3a	0b	13a	22a	12a
Seashore Paspalum	*Paspalum vaginatum*					
	Mauna Kea	3a	100a	100a	100a	98a
	PI364981	2a	38b	48b	77a	92a
Creeping Bentgrass	*Agrostis stolonifera*					
	PennA4	0a	0b	30b	62a	52a
	PI221906	0a	78a	85a	77a	83a

† Species means (within rows) at different herbicide rates followed by the same letter are not considered statistically different according to Fisher's protected LSD at α 0.05.

Table 3-3. Fenoxaprop applicability chart for wild type (WT) and herbicide resistant (+M) turf systems. Columns represent species used as turfgrass species and rows indicate weed species. WT columns indicate current potential control of each weed species using fenoxaprop. +M columns indicate potential control of each weed species using fenoxaprop if the turfgrass species cultivar was developed with one or more site of action mutations conferring resistance to fenoxaprop.

Weed Species	**Turfgrass Species**	Creeping bentgrass	*Agrostis stolonifera*	Colonial bentgrass	*Agrostis capillaris*	Tall fescue	*Festuca arundinacea*	Sheep fescue	*Festuca ovina*	Red fescue	*Festuca rubra*	Annual ryegrass	*Lolium multiflorum*	Perennial ryegrass	*Lolium perenne*	Annual bluegrass	*Poa annua*	Texas bluegrass	*Poa arachnifera*	Kentucky bluegrass	*Poa pratensis*	Buffalograss	*Bouteloua dactyloides*	Common bermudagrass	*Cynodon dactylon*	African bermudagrass	*Cynodon transvaalensis*	Hybrid bermudagrass	*Cynodon hybrid*	Centipedegrass	*Eremochloa ophriuroides*	Seashore paspalum	*Paspalum vaginatum*	St. Augustinegrass	*Stenotaphrum secundatum*	Zoysiagrass	*Zoysia japonica*	Zoysiagrass	*Zoysia Matrella*
Common Name	**Species**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**	**WT**	**+M**
Creeping bentgrass	*Agrostis stolonifera*	N†	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Colonial bentgrass	*Agrostis capillaris*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tall fescue	*Festuca arundinacea*	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Sheep fescue	*Festuca ovina*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Red fescue	*Festuca rubra*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Annual ryegrass	*Lolium multiflorum*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Perennial ryegrass	*Lolium perenne*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Annual bluegrass	*Poa annua*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Texas bluegrass	*Poa arachnifera*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Kentucky bluegrass	*Poa pratensis*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Buffalograss	*Bouteloua dactyloides*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Common Bermudagrass	*Cynodon dactylon*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
African bermudagrass	*Cynodon transvaalensis*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Hybrid bermudagrass	*Cynodon hybrid*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Centipedegrass	*Eremochloa ophriuroides*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Seashore paspalum	*Paspalum vaginatum*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
St. Augustinegrass	*Stenotaphrum secundatum*	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Zoysiagrass	*Zoysia japonica*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Zoysiagrass	*Zoysia matrella*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Southern crabgrass	*Digitaria ciliaris*	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Dallisgrass	*Paspalum dilatatum*	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Goosegrass	*Eleusine indica*	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Knotgrass	*Paspalum distichum*	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y
Bahiagrass	*Paspalum notatum*	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y

† N indicates no control while Y indicates control.

Chapter 4

DISCUSSION AND CONCLUSIONS

Resistance to ACCase inhibiting herbicides has been reported in 42 species worldwide (Heap, 2012) in both weedy grasses as well as species used as turfgrass. Some turfgrass species have high levels of resistance to one or more ACCase inhibiting herbicides and in some cases those herbicides are registered for weed control in these species. With the high levels of resistance reported in these species, it is logical to consider that a site of action point mutation in the target enzyme of the herbicide could be the source of the resistance. Our research results indicate the resistance is not due to the presence of a resistance mutation at any of the seven known sites for centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), bermudagrass (*Cynodon* spp.), tall fescue (*Festuca arundinacea* Schreb.), Kentucky bluegrass (*Poa pratensis* L.), and zoysiagrass (*Zoysia* spp.). The resistance observed in centipedegrass, bermudagrass, tall fescue, Kentucky bluegrass, and zoysiagrass is most likely due to a mechanism such as metabolic resistance or possibly an unreported site of action mutation.

Metabolic resistance refers to the plant's ability to metabolize the herbicide to a less toxic or even a non-toxic form. Metabolic resistance to ACCase inhibiting herbicides has been reported in many species but is not well understood and normally provides only moderate levels of resistance (Preston et al., 1996; Romano et al., 1993; Vila-Aiub et al., 2005).

Site of action mutations were found in three of the 24 species surveyed. The resistance mutation 1781 Ile to Leu was confirmed for red fescue (*Festuca rubra* L.) which was previously reported by Delye and Michel (2005). The 1781 Ile to Leu mutation was also found in sheep fescue (*Festuca ovina* L.). This species was previously reported to contain a resistant form of ACCase (Catanzaro et al., 1993), but the nature of the resistant ACCase was unknown until this research. Annual bluegrass (*Poa annua* L.) was confirmed to contain the 1781 Ile to Leu resistance mutation reported by Delye and Michel (2005).

In Chapter 3 of this research, fenoxaprop resistance was confirmed in all species *(Festuca rubra, F. ovina* and *Poa annua)* containing the 1781 Ile to Leu mutation. There are, however, species expressing a high level of resistance to fenoxaprop where previously reported site of action mutations were not present. Colonial bentgrass (*Agrostis capillaris* L.), annual ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), dallisgrass (*Paspalum dilatatum* Poir.), and zoysiagrass (*Zoysia japonica* Steud. and *Z. matrella* (L.) Merr.) were categorized as resistant in Chapter 3. The resistance in these species is not due to the presence of a mutation at any of the seven mutation sites surveyed in Chapter 2 excluding 1781 for *Z. matrella*, which did not have sequence data generated. The source of resistance for these species is likely due to herbicide metabolism or some other unknown form of resistance as previously discussed.

There was one single nucleotide polymorphism (SNP) that was found in all *Cynodon* species that could possibly be the source of resistance to diclofop-methyl in bermudagrass. Of the 24 grass species studied, only bermudagrass contained a SNP at position 1755 encoding for the amino acid glutamine. Further research is needed to

determine if this SNP is the source of resistance to diclofop-methyl in bermudagrass. While there were numerous other SNPs found for species, none of these SNPs were associated with resistance to ACCase inhibiting herbicides.

The primary goal of this research was to gain new information regarding herbicide response and the presence of herbicide resistance conferring mutations in turfgrass and weedy grass species. This new information can be used to focus efforts for development. Susceptible turfgrass species can have resistant genotypes developed and planted to introduce herbicide selectivity for the removal of susceptible grass species by ACCase herbicide application. High levels of resistance can be observed in genotypes where there are no previously reported resistance conferring mutations present. Therefore the physiological response to the ACCase herbicide to be used in the system should be understood for all genotypes.

Information gained from both aspects of this research, mutation detection and resistance levels to ACCase inhibitors, are valuable in developing effective herbicide resistance weed control systems in multiple turfgrass species. Development of new cultivars containing mutations conferring resistance to ACCase inhibiting herbicides have the greatest utility in susceptible species such as hybrid bermudagrass, seashore paspalum (*Paspalum vaginatum* Sw.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) where control of susceptible weedy grasses such as common bermudagrass and goosegrass (*Eleusine indica* (L.) Gaertn.) is currently not possible with available herbicides. These cultivars will greatly improve management of problematic grassy weeds and make the conversion from one species to another species (for example hybrid

bermudagrass to seashore paspalum) much more efficient by utilizing ACCase inhibiting herbicides through the conversion process.

LITERAURE CITED

Catanzaro C.J., Burton J.D., and Skroch W.A. 1993. Graminicide resistance of acetyl-coA carboxylase from ornamental grasses. Pestic. Biochem. Physiol. 45:147-153.

Delye C. and Michel S. 2005. 'Universal' primers for PCR-sequencing of grass chloroplastic acetyl-CoA carboxylase domains involved in resistance to herbicides. Weed Res. 45:323-330.

Heap I.M. 2012. International survey of herbicide-resistant weeds. Weed Science. http://www.weedscience.org (March 21, 2012)

Preston C., Tardif F.J., Christopher J.T., and Powles S.B. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochem. Physiol. 54:123-134.

Romano M.L., Stephenson G.R., Tal A., and Hall J.C. 1993. The effect of monooxygenase and glutathione-S-tranferase inhibitors on the metabolism of diclofop-methyl and fenoxaprop-ethyl in barley and wheat. Pesticide Biochem. Physiol. 46:181-189.

Vila-Aiub M.M., Neve P., and Powles S.B. 2005. Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. New Phytol. 167:787-796.

APPENDIX A. List of accessions obtained from the USDA, National Plant Germplasm System including accession ID, the form of material received, and the country of origin of each accession (USDA, 2012).

Accession ID	Form	Origin
Agrostis capillaris		
W6 18294	Seed	China
PI 283173	Seed	Czechoslovakia
PI 311011	Seed	Romania
PI 478595	Seed	United Kingdom
PI 234685	Seed	Denmark
Agrostis stolonifera		
PI 221906	Seed	Afghanistan
PI 251945	Seed	Austria
PI 235440	Seed	Switzerland
PI 204390	Seed	Turkey
PI 578530	Seed	USA
Bouteloua dactyloides		
PI 421717	Seed	USA
Cynodon dactylon		
PI 288221	plant	Madagascar
PI 224141	plant	South Africa
PI 287151	plant	India
PI 297827	plant	Israel
PI 287151	seed	India
Cynodon hybrid		
PI 564242	plant	Zimbabwe
Cynodon transvaalensis		
PI 289923	plant	South Africa
PI 286584	plant	India
PI 291981	plant	Ethiopia
PI 615161	plant	Israel
PI 647878	plant	Australia
Digitaria ciliaris		
PI 300938	seed	Zaire
Eleusine indica		
PI 442480	seed	Belgium
PI 408802	seed	China
PI 217609	seed	India
PI 315700	seed	South Africa
PI 226270	seed	Zimbabwe
Eremochloa ophiuroides		
PI 414363	plant	Hong Kong

PI 452430	plant	China
PI 647882	plant	China
PI 647883	plant	China
Festuca arundinacea		
PI 598923	seed	Italy
PI 224974	seed	South Africa
PI 655116	seed	Morocco
PI 234892	seed	Switzerland
PI 203728	seed	Uruguay
Festuca ovina		
PI 312453	seed	Former Soviet Union
PI 380849	seed	Iran
PI 236832	seed	Canada
PI 234750	seed	Spain
PI 595178	seed	China
Festuca rubra		
PI 212299	seed	Afghanistan
PI 234899	seed	Switzerland
PI 349063	seed	USA
PI 598731	seed	Argentina
W6 23532	seed	China
Lolium multiflorum		
PI 632537	seed	USA
PI 162678	seed	Uruguay
PI 222526	seed	Iran
PI 410154	seed	South Africa
PI 251826	seed	Australia
Lolium perenne		
PI 577251	seed	Serbia and Montenegro
PI 420127	seed	Japan
W6 20490	seed	Washington
PI 381113	seed	Netherlands
PI 462336	seed	New Zealand
Paspalum dilatatum		
PI 274081	seed	Swaziland
PI 202300	seed	Chile
PI 419931	seed	Japan
PI 222812	seed	Iran
PI 202190	seed	Argentina
Paspalum distichum		
PI 508731	seed	Argentina
PI 403999	plant	Australia

PI 222796	seed	Iran
PI 284500	seed	Ireland
PI 364977	seed	South Africa
Paspalum notatum		
PI 508823	seed	Brazil
PI 508827	seed	Argentina
PI 508843	seed	Bolivia
PI 508847	seed	Argentina
PI 508850	seed	Argentina
Paspalum vaginatum		
PI 647891	plant	Israel
PI 647900	plant	Thailand
PI 647902	plant	Australia
PI 364981	plant	South Africa
Poa annua		
PI 236900	seed	Canada
PI 442543	seed	Belgium
PI 659870	seed	Germany
W6 28171	seed	Germany
Poa arachnifera		
PI 632500	seed	USA
W6 24210	seed	USA
W6 17708	seed	USA
PI 655088	seed	USA
Poa pratensis		
W6 23480	seed	China
W6 25762	seed	Iceland
W6 28083	seed	Czech Republic
W6 21534	seed	Mongolia
PI 659878	seed	Germany
Stenotaphrum secundatum		
PI 509038	plant	Argentina
PI 410363	plant	South Africa
PI 289729	plant	Madagascar
PI 291594	plant	Zimbabwe
Zoysia japonica		
PI 231389	plant	Japan
PI 324184	plant	South Korea
PI 553016	plant	China
Zoysia matrella		
PI 231146	plant	China
PI 264343	Plant	China