Biology and control of yellow foxtail and knotroot foxtail

by

Mikerly Mistral Joseph

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 6, 2023

Key words: control, *Setaria, DNA barcoding, foxtail*

Approved by

J. Scott McElroy, Ph.D., Chair, Professor of Crop, Soil, and Environmental Sciences
David Han, Ph.D., Associate Professor of Crop, Soil, and Environmental Sciences
David Russel, Ph.D., Assistant Professor of Crop, Soil, and Environmental Sciences

Abstract

Setaria pumila Roem. & Schult. (1817 and *Setaria parviflora* (Poir.) Kerguelen, respectively known as yellow and knotroot foxtail, are two common species infesting turfgrass, pastures, roadsides, and some cropping systems in the United States with few herbicidal control options. Research was conducted in greenhouse and field to evaluate the response of yellow and knotroot foxtail to different herbicides. Results of the greenhouse studies showed that all the treatments control yellow foxtail >87% in a single post-application**.** In contrast, only sulfentrazone alone controlled knotroot foxtail >90% resulting in a complete reduction of above-ground biomass. Sethoxydim (520 g ha-1), metribuzin, and imazaquin controlled knotroot foxtail >70% at 28 DAA. Field studies were more variable. Our results indicate that knotroot foxtail is more difficult to control, making differentiation of these two species important before herbicides are applied.

Yellow and knotroot share morphological characteristics and are often misidentified, which may result in improper herbicide selection. The criteria to differentiate yellow and knotroot appears late, long after identification is required for weeds management in turfgrass. Research was conducted at the herbicide resistance diagnostics laboratory at Auburn University in Alabama to differentiate yellow and knotroot foxtail beyond morphological characteristics using DNA barcoding. Yellow foxtail closely resembles knotroot foxtail, and standard measurements of culm lengths, blade widths, panicle, and floret lengths often overlap between species making identification a challenge. No single character taken on its own is sufficient for distinguishing the two species. It demands close evaluation of multiple characters at once. Sanger sequencing results of *rbcl, ITS, and matK* regions demonstrate the presence of different single nucleotide polymorphisms in the sequenced regions that can differentiate yellow and knotroot foxtail. The Neighbor-joining phylogenies using concatenate sequence of *ITS, rbcl and matk* demonstrate a close evolutionary relationship between yellow and knotroot foxtail. The bootstrap analyses support a monophyletic origin of yellow and knotroot foxtail (i.e. 90%). All the foxtail biotypes collected were clustered into their respective clades. Some foxtail biotypes, classified as unknown, were clustered either on yellow or knotroot foxtail using, making those barcodes relevant for differentiating the foxtail at early stages.

Acknowledgments

This work is the fruit of a long journey with the accompaniment of multiple individuals. I want to thank my advisor, Dr. Scott McElroy, for his proper guidance, valuable advice, and endless support throughout this master's. I want to thank my committee members, Dr. David Russel and David Han, for their guidance. I also express my gratitude to my fellow graduate student and collaborators: Manisha Parajuli, Annu Kumari, Claudia A. Rutland, Bridgette Johnson, and Dr. Jinesh Patel, for their support and assistance.

I also express my sincere appreciation and thanks to my family and friends in Haiti. I am tremendously grateful to my friends: Saint Preux Jean Fritz, Marc Roldy Presumé, Angelo Belizaire, Christelle Yardley, Lucianna Sainclaire, Guy Wolkynn Pierre Louis. I would like to thank my fiancé, Chimeldine Servius, for her support, patience, and love while in graduate school.

Table of Contents

List of Tables

List of Figures

List of abbreviations

ABGR	Above ground biomass relative to the non-treated
ai	Active ingredients
Al	Alabama
C	carbon
^{o}C	Celsius
CA	California
Cbol	The Consortium for the Barcode of Life
DAA	Days after application
DE	Delaware
I_{50}	Rate giving 50% control of the plants
G	gram
ha	hectare
Kg	Kilogram
µl	Micro liters
mL	Milliliters
NC	North Carolina
NJ	Neighborhood join
PA	Pennsylvania
WR_{50}	Value giving 50% reduction of the biomass

Chapter 1

Literature Review

The turfgrass industry in the United States is an important market because of its environmental and aesthetic benefits (Yi and Duffy, 2012). Those benefits include erosion control, increase in water infiltration, reduction of nutrient leaching, carbon (C) sequestration, and aesthetic value improvement of properties (Beard and Green, 1994). Turfgrass is extensively used in many places, such as roadsides, golf courses, and home lawns which is associated with increased demand for aesthetic perfection (Walsh et al., 1999). Such interest in turf aesthetic value is correlated with the need for efficient weed control as weeds infest turf, reduce quality, and compete for light, nutrients, and water (Brosnan et al., 2020).

Morphology, germination, physiology of growth, competitive ability, and reproductive biology are essential weed biology knowledge for developing both economically and environmentally weed management systems (Bhowmik, 2017). Weed identification and correct herbicide selection constitute the basis of efficient management programs. Selective herbicides may result in limited or no control without proper identification (Naidu, 2012). However, misunderstanding of morphological and botanical traits may happen in close species, resulting in improper herbicide selection (Naidu, 2012).

Setaria pumila (Poir.) Roem`. & Schult. (1817) and *Setaria parviflora* (Poir.) Kerguelen, respectively known as yellow and knotroot foxtail, are two common species infesting turfgrass, pastures, roadsides, and some cropping systems in the southeastern region of the United States. Yellow and knotroot foxtail belong to the genus *Setaria*, which contains other major weeds such as *Setaria faberi* Herrm. (1910), and *Setaria viridis* (L.) P. Beauv, respectively known as giant and green foxtail (Dekker, 2003). Although there is no published phylogenetic analysis of *Setaria,* some speculations affirm that Africa is the center origin of the genus (Dekker, 2003). For instance, 74 out of 125 species are found in Africa, and genomic evidence of tropical origin supports this conclusion (Wang, 1994).

Setaria taxonomy is complex due to the morphological similarities of these species (Dekker, 2003). The high degree of overlapping morphological characters and the diverse polyploidy level within and between species complicates the genus classification (Darmency and

Dekker, 2011). However, some evidence demonstrates that the genus *Setaria* is divided into two groups or clades based on developmental characteristics and genome structure (Kellogg et al., 2009). The first class respectively includes yellow and knotroot foxtail (Kellogg et al., 2009). The second one contains foxtail millet, giant, bristly, and green foxtail (Kellogg et al., 2009). Other studies have suggested categorizing *Setaria* into three major groups based on the type of inflorescence, consisting of spiciform panicles, open panicles, and unilateral racemes along the panicles (Kellogg et al., 2009). Systematic relationships among the weedy foxtails are poorly understood, and no explicit phylogenetic hypotheses have been advanced for this group. Arguably, yellow foxtail and knotroot foxtail are more closely related than foxtail millet, green foxtail, or giant foxtail (Wang et al., 1995).

According to Kellogg et al. (2009), *Setaria* is conventionally circumscribed in the bristle clade. The bristle clade includes annual or perennial plants (Kellogg et al., 2009). Their description botanic is as follows:

"*leaf blades flat, folded, or plicate; inflorescences are open or speciform, panicles with spikelets placed along primary branches; spikelets are usually dorsally compressed and planoconvex, all or some spikelets subtended by one or more bristles that persist on the axis when the spikelets fall maturity; the glumes are unequal*" (Kellogg et al., 2009).

Overall, the *Setaria* is a genus that pertains to the tribe Panicea, the family Gramineae and the subfamily of Panicoidae (Rominger, 1959). It contains approximately 125 species dispersed throughout temperate, tropical, and subtropical regions (Rominger, 1959). Some *Setaria* species are domesticated as cereal crops, such as foxtail millet [Setaria. *italica* (L.) P. Beauv.] but the vast majority are wild species (Morrone et al., 2014; Santelmann et al., 1963; Wang, 1994).

Diversification within the genus Setaria

The *Setaria* genus has one of the smallest genome sizes among the cereals but has a tremendous intraspecific variation within the species (Wang, 1994). Foxtails vary in chromosome number and morphological characteristics within the species (Wang, 1994). Foxtail's karyotypes differ depending on the species (Darmency and Dekker, 2011). For instance, the species green and millet foxtail contain 2x chromosome base, whereas giant foxtail has 4x chromosome bases (Darmency and Dekker, 2011). Other foxtails may have 6x, or 8x chromosomes base possible (Darmency and Dekker, 2011). This genomics differentiation has led to extensive research to

understand the diversity within species and between species and helps in the taxonomy of *Setaria* (Zhao et al., 2013a).

One of the critical observations of *Setaria* behavior is the presence of phenotypic heterogeneity instead of genetic diversity among the individuals of a population (Dekker, 2003; Wang, 1994). Significant intraspecific variation in morphology within the *Setaria* is observed, whereas some traits are in natural environmental or other heritable variation (Wang, 1994). Generally, those individual traits vary within the species: colors of leaves, panicles and bristles, panicle shape, seed, and panicle size, along with others (Rominger, 1959). For example, several morphological variants have been reported inside the foxtail millet, such as divided segmented panicles, even if these traits are nonsexual (Darmency and Dekker, 2011). In addition, the number and position of bristles in the inflorescence vary considerably among *Setaria* species, and those traits are commonly used to discriminate grass species (Kellogg, 2017). Those morphological variations within species may evolve confusion in the identification of the species. However, panicle length or size, leaf shape, and pubescence are commonly used in the identification of foxtail (Dekker, 2003). While those identification criteria may be helpful, yellow and knotroot foxtail are frequently misidentified by weed managers since they share similarities (Chikara and Gupta, 1980; Darmency and Dekker, 2011; Dekker, 2003).

Biology and ecology of yellow and knotroot foxtail

Origin and distribution.

Yellow foxtail [Setaria *pumila* (Poir.) Roem. & Schult. (1817)] and knotroot foxtail [Setaria *parviflora* (Poir.) Kerguelen] are both weedy species, originating respectively in Eurasia and the north America (Rominger, 1959). Some phylogenetics analysis place yellow foxtail with African *Setaria* species, suggesting that yellow foxtail is native to Africa (Kellogg et al., 2009). Over the years, yellow foxtail has become a ubiquitous weed, and it has been inferred that yellow foxtail propagation is due to pre-Columbian dispersal from the old world to the new world (Dekker, 2003). Knotroot foxtail is the most widespread indigenous *Setaria* in the western hemisphere (Rominger, 1959). It probably spread from South America to North America (Dekker, 2003). Nevertheless, the propagation mechanism is doubtful, and more research are needed to confirm the precise origin point and expanding of those species (Dekker, 2003; Wang, 1994). Yellow foxtail and knotroot originated from two different continents; nevertheless, they share similarities

(Darmency and Dekker, 2011). Their differences in terms of origin and their phylogenetic relationship present an intriguing enigma, and little research has been done to understand the potential gene flow between the two species (Rominger, 1959; Schröder et al., 2016). It has been suggested that the two species share a common African ancestor, and that their divergence from this common ancestor occurred relatively recently(Wang, 1994).

The population genetic structure of yellow foxtail consists of three nearly distinct clusters: Eurasia, the northern United States, and the southern United States (Darmency and Dekker, 2011). Yellow foxtail is widely distributed in the United States, Argentina and Uruguay, Africa, the Middle East, Europe, Asia, Australia, and many Pacific islands. Knotroot foxtail distributed throughout the Americas, southern Africa, most of Europe, the Middle East, Asia, Australia, and several Pacific islands. In the United States, knotroot foxtail occurs along the Atlantic coast from Massachusetts to Florida, the Gulf Coast from Florida to Texas, and the Carolinas westward to Kansas and California (Hitchcock, 1971; Rominger, 1959).

Botanical description

Yellow foxtail is botanically described as:

"Roots fibrous; culms usually erect, solitary, or in clumps of several tillers and branching at the base with nodes sometimes geniculate below, mostly 10-120 cm tall; leaf sheaths without hairs on margins; ligule a fringe of ciliate hairs joined at the base, to 3 mm high, with about 50 cilia per mm; leaf blades 4-10 mm wide, to 30 cm long, loosely twisted, scabrous on the upper surface with about 80 (50-300) hygroscopic hairs 4-10 mm long just above the ligule; panicles, usually 3-10 cm long; ranches of the panicle less than 1 mm long, bearing one fertile spikelet with a cluster of 4-12 bristles (rarely 3) below it; bristles 3-10 mm long, yellow, orange or tawny at maturity; spikelets thick, planoconvex, 3.0-3.5 mm long; glumes five-nerved, second glume covering about half the coarsely transverse-rugose fertile lemma. Fruit a caryopsis, elliptic in longitudinal section, depressed ovate in cross-section, 2.5-3.3 mm long, 1.5-2.2 mm wide, 1.0-1.5 mm thick, articulating below the glumes, remaining enclosed within the hardened lemma and palea (Hitchcock, 1971; Morrone et al., 2014; Steel et al., 1983).

Knotroot foxtail in its counterpart is described botanically as:

"*A perennial plant, rhizomatous, fibrous-rooted; culms usually arise singly or in small tufts; roots of mature plants are fibrous, but also produce short knotty rhizomes; plant tall is about 78-120 cm, culm 0.5 cm thick, leaf blade 14.5 to 18 cm in length and 1 cm wide; blade and sheath are pubescent with hairy ligules but lacking auricle; leaf blades are rough on the upper side and smooth on the lower side. The sheath is smooth, and the ligule is a fringed membrane; the panicle is cylindrical, 0.6 to 2.5 cm wide, 1.27 10.16 to 4 cm long, and yellow to brown or purple in color and densely flowered. Each spikelet has 4 to 12 bristles that are 0.5 to 2.8 cm long* (Bryson and DeFelice, 2009; Montiel and Mayra, 1975; Rominger, 1959).

Morphological differences and similarities

Yellow foxtail can be differentiated to knotroot foxtail in their life habits (yellow foxtail, annual, and knotroot foxtail perennial), by spikelet length (2 to 2.5 mm for knotroot foxtail, 3 mm for yellow foxtail), the lower floret sexuality (staminate for knotroot foxtail, neuter for yellow foxtail), and palea development (reduced for knotroot foxtail, well developed for yellow foxtail) (Hitchcock, 1971; Rominger, 1962b). Both yellow foxtail and knotroot foxtail are pubescent at the base of the leaf blade (Dyer et al., 2022). Chikara and Gupta (1980) found that those two species have 71% percent of similarities by considering 24 criteria such as plant height, node number, spikelet shape; stomata length and further concluded that those two species could be distinguished only by their annual habit and by larger and turgid spikelets. According to Darmency and Dekker (2011), the primary morphological differences between yellow and knotroot foxtail are found in the spikelet length and the lower floret. However, those criteria are not unanimous for differentiating the species. For instance, Wang et al. (1995) specify that the rhizomes of knotroot foxtail are the only distinctive specific characteristics to yellow foxtail but offer little practical help for identification because they tend to appear late during development or sometimes are not apparent. Therefore, more research is needed to characterize those morphological differences, which can create a framework for proper identification at early stages.

Germination

Seed germination of yellow foxtail begins in early spring; however, the emergence dates vary with location (Milton, 1998). Yellow foxtail seed is reported to have maximum germination at 20 and 25 ^{0}C , but the germination decrease in the temperature range of 25 to 35 ^{0}C (Manthey and Nalewaja, 1987). In addition, yellow foxtail seed did not germinate below 10 ^{0}C and above 40 ^{o}C (Norris and Schoner, 2017). Knotroot foxtail is a perennial grass, and its germination occurs in late spring (Mollard and Insausti, 2009). However, there are few researches about the optimal temperature and timing for knotroot foxtail germination.

Cytogenetics

Yellow foxtail and knotroot foxtail have different polyploidy levels (Doust and Diao, 2017). Yellow foxtail chromosome numbers include the euploid series 2n = 18, 36, 44, and 72, and some aneuploids, with 2n = 36 and 2n = 72 (Rominger, 1959; Steel et al., 1983). Knotroot foxtail chromosome numbers are either 2n = 36 or 2n = 72 (Kellogg, 2017). Both genomes strongly hybridize when using nuclear marker knotted1 implying that these two species have a close genetic relationship (Zhao et al., 2013a). Likewise, Layton and Kellogg (2014) used the chloroplast gene ndhF leading to conclude and confirm the genomics similarities of those species. The pattern followed by the *Setaria* species shows low intra-population genetic diversity and unusually high genetic diversity between populations (Dekker, 2003; Wang, 1994). How do those genetic variations affect the yellow and knotroot foxtail morphology? Do those variations affect the genetic identification of those two species? For now, little research has been done to gain an understanding of those potential genomic variations for knotroot and yellow foxtail populations (Dekker, 2003; Wang, 1994)

Control of yellow and knotroot foxtail

Pre and post-emergence herbicides for selective control of annual grass weeds, including foxtails, have become important in turfgrass management programs (Bhowmik and Bingham, 2017). However, little research has been done to assess yellow and knotroot foxtail response to selective herbicide in turfgrass since they could show opposite responses. Pinoxaden, labeled in the United States for use on bermudagrass, provided postemergence control of yellow foxtail (Peppers et al., 2020). For instance, pinoxaden injured yellow foxtail by more than 95% at rates of

10 g ai ha-1 and higher and reduced the above-ground biomass by more than 90% (Peppers et al., 2020). Similarly, fenoxaprop-P-ethyl + mefenpyr-diethyl provided selective control of yellow foxtail in tall fescue (Zabihollahi et al., 2008). In pastures, chlorsulfuron applied at 0.07 and 0.14 kg/ha gave season-long control of yellow foxtail when applied in late fall or early spring in Kentucky bluegrass (Maloy, 1985). Moreover, imazethapyr applied at 0.07 kg·ha^{1} provides effective control of yellow foxtail with no detrimental effects on buffalograss establishment when applied as a preemergence herbicide (Fry et al., 1997). As pre-emergence, Bhowmik and Bingham (2017) found that dinitroaniline provided residual preemergence control of several annual weeds, including yellow foxtail, during the entire growing season with the labeling dose. Nevertheless, the literature about the control of knotroot foxtail in turfgrass is poor. In Pastures conditions, Bums (2006) found hexazinone at 1.26 kg/ha alone or applied with metsulfuron + dicamba + 2,4-D or chlorsulfuron or nicosulfuron or metsulfuron provided at least 70% knotroot foxtail control at 14 DAA. Imazapic applied at 140 kg ai/ha control knotroot foxtail >80% at 12 DAA regardless of with or without mowing (Coats et al., 1999). However, in a separate field trial, imazapyr applied at the same rate only provided 60% control at 4 weeks after treatment, and control fell below 10% at 8 weeks after treatment (Coats et al., 1999). Pendimethalin applied pre-emergence early in the season and followed by a postemergence application of quinclorac, provided excellent control of annual foxtails including yellow foxtail in established alfalfa, bahiagrass, bermudagrass, and tall fescue pastures. However, pendimethalin and quinclorac are not effective on knotroot foxtail (Flessner et al., 2018). Did yellow and knotroot foxtail show similar responses to the herbicides? Research is needed to address those questions, as yellow and knotroot foxtail are subject to misidentification.

Research objectives

The objective of the thesis is to assess mainly the control options for yellow and knotroot foxtail in managed turfgrass. This thesis is split into two parts. The first study focuses on the control options for yellow in knotroot foxtail using common turfgrass herbicides. The second objective was to differentiate yellow and knotroot foxtail using morphological characteristics and genetic markers.

Literature cited

Beard JB, Green RL (1994) The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans. Journal of environmental quality 23:452-460

Bhowmik PC (2017) Weed biology: importance to weed management. Weed Science 45:349-356

Bhowmik PC, Bingham SW (2017) Preemergence Activity of Dinitroaniline herbicides Used for Weed Control in Cool-Season Turfgrasses. Weed Technology 4:387-393

Brosnan JT, Elmore MT, Bagavathiannan MV (2020) Herbicide-resistant weeds in turfgrass: current status and emerging threats. Weed Technology 34:424-430

Bryson CT, DeFelice MS (2009) Weeds of the south Athens, GA: University of Georgia Press. Pp. 495

Bums BK (2006) Control of invasive grasses Master Mississippi: Mississippi State University 85 p

Chikara J, Gupta PK (1980) Numerical taxonomy in the genus Setaria (L.) Beauv. Indian academic Plant Science 89:401-406

Coats GE, Taylor JM, Kelly ST, Helms RB, Scott TD (1999) Highway vegetation management Mississippi: Miss. Agric. and Forest. 122 p

Darmency H, Dekker J (2011) Setaria. Pages 275-291 *in* Kole C, ed. Wild Crop Relatives : Genomic and Breeding Resources. Clemson: Springer

Dekker J (2003) The foxtail (Setaria) species-group. Weed Science 51:641-656

Dyer LM, Henry GM, McCullough PE, Belcher J, Basinger NT Knotroot Foxtail [Setaria parviflora (Poir.) Kerguélen]:"A sly fox". Weed Technology:1-22

Flessner M, Rector L, Pittman K (2018) Control of common grassy weeds
in pastures and hayfields. Virginia Virginia Tech

Fry JD, Gaussoin RE, Beran DD, Masters RA (1997) Buffalograss Establishment with Preemergence Herbicides. Hortscience 34

Hitchcock AS (1971) Manual of the grasses of the United States. New york: Dover

Kellogg Elizabeth A (2017) Evolution of Setaria *in* Doust AN, Diao X, eds. Genetics_and_Genomics_of_Setaria. Switzerland: Springer

Kellogg Elizabeth A, Aliscioni Sandra S, Morrone O, Pensiero J, Zuloaga F (2009) A Phylogeny ofSetaria (Poaceae, Panicoideae, Paniceae) and Related Genera Based on the Chloroplast GenendhF. International Journal of Plant Sciences 170:117-131

Layton DJ, Kellogg EA (2014) Morphological, phylogenetic, and ecological diversity of the new model species Setaria viridis (Poaceae: Paniceae) and its close relatives. Am J Bot 101:539-57

Leguizamon ES, Rodriguez N, Raneiro H, Perez M, Perez L, E. Z, Quintanilla FC (2008) Modelling the emergence pattern of six summer annual weed grasses under no tillage. Weed Research 49:98-106

Maloy BM (1985) Selective control of perennial grasses in Kentucky

blue-grass (Poa pratensis L.) turf with chlorsulfuron. Master of science Iowa Iowa State University

Manthey DR, Nalewaja JD (1987) Germination of Two Foxtail (Setaria) Species. Weed Technology 1:302-304

Milton J-H (1998) Characterization of foxtail (Setaria spp.) seed production and

giant foxtail (S. faberi) seed dormancy at abscission. Doctor of Philosophy Ames: Iowa State University

Montiel L, Mayra B (1975) Comparación anatómico-taxonómica de Setaria magna Griseb.

y Setaria geniculata (Lam .) Beauv. de Florida, EE. UU. Biologia tropical 24:95-197

Morrone O, Aliscioni Sandra S, Fritz VJ, Pensiero J, Zuloaga F, Kellogg Elizabeth A (2014) A revision of the Old World species of Setaria..pdf. Systematic botany monograph 93-95

Naidu VSGR (2012) Hand Book on Weed Identification. Jabalpur: Directorate of Weed Science Research,

Norris RF, Schoner CA (2017) Yellow Foxtail (Setaria lutescens) Biotype Studies: Dormancy and Germination. Weed Science 28:159-163

Peppers JM, Gonçalves CG, McElroy JS (2020) Rate response of select grass weeds to pinoxaden. Weed Technology 34:818-823

Rominger JM (1959) taxonomy and systematic of setaria. Doctor of Philosophy. Illinois: University of illinois 250 p

Santelmann PW, Meade JA, Peters RA (1963) Growth and developement yellow foxtail and giant. Cambridge University Press 11:139-142

Schröder S, Bahri BA, Eudy DM, Layton DJ, Kellogg EA, Devos KM (2016) Genetic diversity and origin of North American green foxtail [Setaria viridis (L.) Beauv.] accessions. Genetic Resources and Crop Evolution 64:367-378

Steel MG, Cavers. PB, Lee SM (1983) The biology of canadian weeds. Setaria glauca (L.) Beauv. and S. verticillata (L.) Beauv. Canadian journal of plant science 711-725

Walsh B, S. IS, J. B (1999) Biology and Management of Dollar Spot (Sclerotinia homoeocarpa); an Important Disease of Turfgrass. Hortscience 34:13

Wang R-L (1994) weedy adaptation in setaria spp: Genetic diversity, population genetic structure and variation in herbicide resistance. Doctor of Philosophy. Iowa: Iowa State Univeristy. 124 p

Wang RL, Wendel JF, Dekker JH (1995) Weedy adaptation in Setaria spp II Genetic diversity and population genetic. American Journal of Botany 82:1031-1039

WSSA (2007) Herbicide Handbook Lawrence: Weed Science Society of America

Yi J, Duffy PA (2012) Economic Analysis of Turfgrass-Sod Production in Alabama. Pages 1-92

Zhao M, Zhi H, Doust AN, Li W, Wang Y, Li H, Diao X (2013) Novel genomes and genome constitutions identified by GISH and 5S rDNA and knotted1 genomic sequences in the genus Setaria. BNC Genomic

Chapter II.

Herbicides control options of *Setaria pumila* (yellow foxtail) and *Setaria parviflora* (knotroot foxtail) for possible use in turfgrass

Abstract

Setaria pumila (Poir.) Roem. & Schult. (1817) and *Setaria parviflora* (Poir.) Kerguelen, respectively known as yellow and knotroot foxtail, are two common weeds species infesting turfgrass and pastures in the southeastern region of the United States. Yellow and knotroot foxtail share morphological similarities and are frequently misidentified by weed managers, thus leading to confusion in herbicide selection. Research was conducted in greenhouse and field to evaluate the response of yellow foxtail and knotroot foxtail to several turfgrass herbicides. The evaluated herbicides were: Pinoxaden (35 and 70 g ha^{-1}), sethoxydim (316 and 520 g ha^{-1}), thiencarbazone+dicamba+iodosulfuron (230 g ha^{-1}), nicosulfuron+rimsulfuron (562.8 g ha^{-1}), metribuzin (395 g ha^{-1}), sulfentrazone (330 g ha^{-1}), sulfentrazone+imazethapyr (504 g hai^{-1}), imazaquin (550 g ha^{-1}). In greenhouse studies, all the treatments control yellow foxtail >87%**.** By comparison, only sulfentrazone alone controlled knotroot foxtail > 90% resulting in complete reduction of above-ground biomass. Sethoxydim (520 g ha^{-1}), metribuzin, and imazaquin controlled knotroot foxtail >70% at 28 DAA. Field studies were more variable. In a rate response evaluation, yellow foxtail was approximately eight times more susceptible to pinoxaden and two times more susceptible to sethoxydim than knotroot foxtail based on log(I_{50}). Our research indicates that knotroot foxtail is more difficult to control across a range of herbicides making differentiation of these two species important before herbicides are applied.

Additional index words: Yellow, knotroot, foxtail, control, turfgrass

Nomenclature: Setaria *pumila* (Poir.) Roem. & Schult. (1817), Setaria *parviflora* (Poir.) Kerguelen

Introduction

In the southeastern region of the United States, *Setaria pumila* (Poir.) Roem. & Schult. (1817) and *Setaria parviflora* (Poir.) Kerguelen, respectively known as yellow and knotroot foxtail, are two common species infesting managed and unmanaged turfgrass, pastures, roadsides, and some cropping systems (Bryson and DeFelice, 2009; Hitchcock, 1971). Yellow and knotroot foxtail belong to the genus *Setaria* which contains other major weeds such as *Setaria faberi* Herrm. (1910), and *Setaria viridis* (L.) P. Beauv. respectively known as giant and green foxtail. Yellow and knotroot foxtail originated from Asia and North America, respectively (Dekker, 2003; Rominger, 1962a). Nevertheless, they share morphological similarities and are frequently misidentified by weed managers, thus leading to confusion in the herbicide selection (Darmency and Dekker, 2011). The primary morphological differences between yellow and knotroot foxtail are found in the seedhead with yellow foxtail seedheads bigger and denser with more hairs (Darmency and Dekker, 2011). Other studies specify that the rhizomes of knotroot foxtail are the only distinctive specific characteristic to yellow foxtail (Wang et al., 1995). However, those criteria are not unanimous for differentiating and offer little practical help for identification as these characteristics appear late during development or sometimes are not apparent (Wang et al., 1995)

Yellow and knotroot foxtail are annual and perennial weeds, respectively, with few options for effective chemical control in warm-season turfgrass. Pinoxaden labeled in the United States for use on bermudagrass controls of yellow foxtail post emergence (Anonymous, 2018; Peppers et al., 2020). For instance, pinoxaden injures yellow foxtail by more than 95% at 0.001 kg ai ha^{-1} and higher rates. It reduces the above-ground biomass by more than 90% but pinoxaden is not labeled to control knotroot foxtail (Peppers et al., 2020). Chlorsulfuron applied at 0.07 and 0.14 kg ha^{-1} gave season-long control of yellow foxtail when applied at the early growth stage in Kentucky bluegrass, but it is not labeled in turfgrass (Maloy, 1985). Imazethapyr applied at 0.07 kg·ha^{1} controls yellow foxtail effectively as a preemergence with no detrimental effects on grass establishment (Fry et al., 1997). http://graduate.auburn.edu/faculty-staff/automated-form-workflows/checking-status-of-smartsheet-workflows/

Little research has been done to gain an understanding of the chemical control of knotroot foxtail. In pasture conditions, hexazinone at 1.26 kg ha^{-1} alone or applied with metsulfuron +

dicamba + 2,4-D, chlorsulfuron or nicosulfuron controlled knotroot foxtail by more than 70% two weeks after application (Coats et al., 1999). Nicosulfuron + metsulfuron applied at 0.04 kg ha^{-1} control knotroot foxtail 70% in bermudagrass forage at the actively growing stage (Rusell, 2021). Other herbicides could potentially control yellow and knotroot foxtail but are not currently labeled. For instance, thiencarbazone+dicamba+iodosulfuron is labeled for controlling yellow foxtail and giant foxtail but not knotroot foxtail.

The objectives of this research were to (1) evaluate the response of yellow foxtail and knotroot foxtail to several turfgrass herbicides and (2) evaluate the rate response of yellow and knotroot foxtail to increasing rates of pinoxaden and sethoxydim and estimate the application rate at which 50% (I_{50}) of both species was injured using a non-linear regression model.

Material and Methods

Research was conducted in 2021 and 2022 in greenhouse and naturalized field populations to evaluate yellow and knotroot foxtail response to different turfgrass herbicides. Three different studies were conducted (1) initial greenhouse herbicide evaluation, (2) field studies, and (3) rate response evaluation of sethoxydim and pinoxaden. All herbicide treatments in both greenhouse and field trials were applied with a CO_2 pressurized sprayer, calibrated to deliver 280 L ha^{-1} with a handheld four-nozzle boom (TeeJet TP8002 flat fan nozzles with 25 cm spacing; Spraying Systems Company, Wheaton, IL). A nonionic surfactant (Induce, Helena®, Chemical Company, Collierville, TN) was included in all treatments at 0.25% v/v. Treatments were compared with a non-treated control.

Initial greenhouse evaluations. Two experiments were conducted at the Auburn University Weed Science greenhouse in Auburn, AL (32.35°N, 85.29°W) in 32/28 C (+/-1 C Day/night) conditions with an average relative humidity of 70%. Seeds of both species were harvested from a local population in Montgomery, Alabama. Seeds were cleaned and stored at 4 °C prior to the experiments. Seeds were planted in flats of potting medium and were then transplanted individually at three leaves stage into 230 cm^3 pots, filled with sandy soil. Pots were irrigated three times a day with overhead irrigation and fertilized (28-8-16 Miracle-Gro Water-Soluble All-Purpose Plant Food, Scotts Miracle-Gro Products Inc, Marysville, OH.) once a week to promote

growth as needed until the plants were healthy and established. Treatments were applied two weeks after transplantation. After treatment, pots were not watered for approximately 24 hours to allow for adequate leaf absorption. Herbicides included: Pinoxaden (35 and 70 g ha^{-1}) (Manuscript®, Syngenta Crop Protection, LLC, Greensboro, NC), Sethoxydim (316, 520 g ha^{-1}) (Segment ™, BASF Corporation, Research Triangle Park, NC), thiencarbazone+dicamba+iodosulfuron (230 g ha^{-1}) (Celsius® WG, Bayer Crop Science, NC), nicosulfuron+rimsulfuron (562.8 g ha^{-1}) (Dupont™Steadfast®, Corteva ™ Agriscience, Wilmington, DE), metribuzin (395 g ha^{-1}) (Sencor®, Bayer Crop Science, NC) , sulfentrazone (330 g ha^{-1}) (Dismiss® CA, FMC Corporation, Philadelphia, PA), sulfentrazone+imazethapyr (504 g ha^{-1}) (Dismiss® South, FMC Corporation, Philadelphia, PA), imazaquin(550 g ha^{-1}) (Scepter T&O, AMVAC Chemical Corporation, Newport Beach, CA).

Field herbicide evaluations. The herbicides applied in the greenhouse were also evaluated in the field. Two separate trials were conducted on a naturalized yellow foxtail population in Tallassee, Alabama, in the summer of 2021 and 2022 in pastures conditions. Yellow foxtail was the dominant forage species, and application was made at seedhead stages. We also evaluated naturalized knotroot foxtail populations established in bermudagrass in two different locations in Auburn and Montgomery, Alabama in the summer of 2021 and 2022 in turf conditions. The plot size for all the field studies was 1.5 by 1.5 m.

Rate Response Evaluation. Due to the various response of the ACCase inhibiting herbicide pinoxaden and sethoxydim, a rate response screen studies for these herbicides were conducted in the greenhouse. Pinoxaden and sethoxydim were applied at 9 different rates to generate a dose response curve. Pinoxaden rates were: 0, 2.21, 4.42, 8.8, 17.7, 35.4, 70.8, 141.4, 282.9, and 565.8 g ha^{-1}. Sethoxydim rates were 0, 19.8, 39.5, 79.0, 158.0, 316.1, 632.2, 1264.0, 2529.0, and 5057.2 g ha^{-1}

Statistical Analysis. All trials were arranged in a randomized complete block design with four replications and were repeated each. Herbicide injury was visibly evaluated to the relative control on a 0% (no phytotoxic effect) to 100% (total plant death) scale at 28 days after application (DAA). The plant was clipped at the soil surface, and fresh above-ground biomass

was recorded at 28 DAA in greenhouse studies. Data were subjected to ANOVA analysis and mean comparison at a significance level of $P<0.05$ using R studio with package dplyr, ggplot2, agricolae, and FSA (Hadley et al., 2019; Ogle et al., 2022; RStudio, 2020). Herbicide, herbicide rate, and run interactions were analyzed with the visible plant injury and relative fresh weight as response variables. Nonlinear regressions were modeled with DRC package in R studio (Ritz et al., 2015; RStudio, 2020). Prior to modeling, nine pinoxaden, and sethoxydim rates were transformed to log rates to maintain equal spacing between treatments, including the non-treated set to 0.04 and 0.99. Both species were modeled with appropriate models that best-expressed plant response with the lowest Akaike Information Criterion and highest lack of fitted test as described by (Knezevic et al., 2007; Seefeldt et al., 2017). Plant visible injury for pinoxaden, sethoxydim, and above-ground biomass for sethoxydim were fitted to a four parameters Weibull equation (Equation 1):

$$f(x) = C + (D - C) \times \exp\{-\exp[b(\log(x) - e)]\}. \quad [1]$$

where f represents the percent, visible injury relative, to the nontreated control, x represents the log-transformed rate, C lower limit, D is the upper limit, b is the relative slope, and e represents the inflection point. This equation was used to calculate the I^{50} and I^{90} values, which are the rate causing 50 and 90% of injuries. Above-ground biomass for Pinoxaden was fitted to a 4 parameters Weibull model (Equation 2):

$$f(x) = C + \frac{(D-C)}{1+[\exp[b(logx-loge)]} \quad [2]$$

where f represents relative percent to the nontreated control, x represents the log-transformed rate, C lower limit, D is the upper limit, b is the relative slope, and e represents the inflection point. This equation was used to calculate the WR^{50} and WR^{90} which are the rate causing 50 and 90% biomass reduction relative to the non-treated. Above-ground biomass data were transformed into relative percentage of the nontreated control using the formula.

$$\%\, Relative = \frac{Mean\ Non\ treated - Mean\ Treatment}{Mean\ Non\ treated} \quad [3]$$

The relationship between the aboveground biomass and visual control was assessed using the Pearson correlation coefficient where 0 is no correlation, 1 is total positive correlation, and –1 is total negative correlation using this fotmula

$$\rho(x, y) = \frac{cov(x,y)}{\sigma_x \sigma_y} \quad [4]$$

Where $\rho(x, y)$ represent the Pearson correlation coefficient, $cov(x, y)$, the covariance between relative above-ground biomass and visual control. σ_x the variance of visual control, and σ_y the variance of relative above-ground biomass(Kotu and Deshpande, 2018).

Results and discussions

Our initial hypothesis was that there is no significant difference in terms of herbicide response between yellow and knotroot foxtail. However, this hypothesis was false based on the results we will present. A significant interaction between runs was not detected based on herbicide evaluation by run interaction for greenhouse studies and yellow foxtail field trials ($P \geq 0.05$); therefore, data were pooled overruns. Nevertheless, a significant interaction between years was detected in knotroot foxtail field trials; therefore, the results are presented separately.

Initial greenhouse. In greenhouse evaluation, yellow and knotroot foxtail responded differently to the selected herbicides. All the herbicides were effective on yellow foxtail with more than 85 % at 28 DAA. Above-ground biomass data followed the same pattern. All the herbicides reduced yellow foxtail above-ground biomass by more than 95% compared to the nontreated at 28 DAA. Knotroot foxtail was more difficult to control in general than yellow foxtail. Sulfentrazone controlled knotroot foxtail > 90 %, which was the best treatment statistically. Similarly, metribuzin controlled knotroot foxtail 81%, imazaquin 71%, sethoxydim (high rate) 76%, and thiencarbazone+dicamba+iodosulfuron 68% control. All the other treatments controlled knotroot foxtail by less than 65%. Relative plant fresh-weight data agreed with visually estimated control data. The Pearson correlation between visual control and relative plant fresh-weight data at 28 DAA were 0.83 and 0.75, respectively for yellow and knotroot foxtail. Sulfentrazone, metribuzin, sethoxydim (high rate), thiencarbazone+dicamba+iodosulfuron and imazaquin reduced the above ground by more than 90% and nicosulfuron+rimsulfuron reduced knotroot foxtail biomass by 89%. However, pinoxaden (low and high rates) were less effective on knotroot foxtail with less than 30% biomass reduction.

Field herbicide evaluations. Yellow foxtail responded differently in the field, with some herbicides providing less control than the greenhouse study. Pinoxaden (high rate), sethoxydim (high rate), and thiencarbazone+dicamba+iodosulfuron controlled yellow foxtail in the field by

more than 70% at 28 DAA. However, pinoxaden (low rate), metribuzin, and imazaquin provided less than 60% control. This differential is understandable because the yellow foxtail plants were at mature stages and in pasture conditions.

Knotroot foxtail evaluation in the field also differed from what was observed in the greenhouse. We detected a significant difference between summer 2021 and 2022 data based on herbicide injury for knotroot foxtail. For the first year, pinoxaden (high rate), thiencarbazone+dicamba+iodosulfuron, and nicosulfuron+rimsulfuron was the best treatment, with more than 50% control at 28 DAA. In parallel, the mean comparison showed no significant difference between pinoxaden (low rate), sethoxydim (low rate), metribuzin, and imazaquin with less than 20% control. However, for summer 2022, pinoxaden (high rate) failed to control knotroot foxtail by more than 20%. Sethoxydim (high rate) and thiencarbazone+dicamba+iodosulfuron provided more than 50% control. The other treatments provided less than 30% control with no significant difference.

Rate response experiment. Yellow foxtail was the most susceptible species for both pinoxaden and sethoxydim. The lack of fitted test was not significant for log-logistic and Weibull model with four parameters, demonstrating a proper choice for estimating I^{50} and WR^{50}. (Ritz et al., 2015). Pinoxaden injured yellow foxtail by more than 80% at 35.36 g ai ha^{-1}; however, at the same rate, pinoxaden injured knotroot foxtail by less than 15 %. The I_{50} values for yellow foxtail visible injury were 6.45 g ai ha^{-1}. Nevertheless, knotroot foxtail susceptibility to pinoxaden was quite different from yellow foxtail. The I^{50} values for knotroot foxtail visible injury were 251.18 g ai ha^{-1}. Visual control agreed with relative biomass data with a Pearson correlation of 0.72. Pinoxaden reduced yellow foxtail biomass by greater than 95% at 8.84 g ai ha^{-1}. The WR^{50} values for yellow foxtail were 1.62 g ai ha^{-1}. In contrast, at 8.84 g ai ha^{-1}, pinoxaden reduced knotroot foxtail biomass by less than 20% and the WR^{50} values estimated for knotroot foxtail biomass reduction were 38.01 g ai ha^{-1}, respectively. Equation parameters and 95% confidence intervals for the visible injury and relative biomass data are displayed in Tables 3 and 4, respectively.

Sethoxydim data followed the same trend as pinoxaden. Sethoxydim injured yellow foxtail by more than 95% at 316.0 g ai ha^{-1}. In contrast, it injured knotroot foxtail by less than 40 percent at the same rate. The I^{50} value estimated were 96 and 2089 g ai ha^{-}, respectively for yellow foxtail and knotroot foxtail. Sethoxydim reduced yellow foxtail biomass by greater than 90% at rates of

79.02 g ai ha^{-1}, and the WR^{50} value estimated for yellow foxtail biomass reduction was 1.62 g ai ha^{-1}. Sethoxydim provided significant reduction of above-ground biomass, but none of the sethoxydim rates injured knotroot foxtail greater than 95 %. It reduced knotroot foxtail biomass by greater than 70% at rates of 316.0 g ai ha^{-1}, and the WR^{50} values estimated for knotroot foxtail biomass reduction was 165.72 ai ha^{-1}.

We detected a significant slope (I^{50}) difference between yellow and knotroot foxtail for both sethoxydim and pinoxaden. This estimation indicated that there was no equal susceptibility between yellow and knotroot foxtail for pinoxaden and sethoxydim. Furthermore, considering the relative biomass, those results indicated that yellow foxtail was eight times more susceptible to pinoxaden than knotroot foxtail and yellow foxtail was approximately two times more susceptible to sethoxydim than knotroot foxtail based on log (WR^{50})

Research Implications. This study found that yellow foxtail responded differently to knotroot foxtail with the selected herbicides, and knotroot foxtail was more difficult to control. Based on the greenhouse studies, sulfentrazone, sethoxydim (high rate) thiencarbazone+dicamba+iodosulfuron, and metribuzin can be considered for controlling yellow and knotroot foxtail at recommended label rate. Knotroot foxtail response was more variable and thiencarbazone+dicamba+iodosulfuron sethoxydim (high rate) can control knotrrot foxtail with not complete suppression. A published report showed that nicosulfuron + metsulfuron, two herbicides inhibiting acetolactate synthase, was one of the best treatments for knotroot foxtail suppression but provided not complete control in bermudagrass hayfield (Bryson and DeFelice, 2009). Our study found nicosulfuron +rimsulfuron, reducing knotroot foxtail biomass by more than 80% in greenhouse studies but with only 33% control in the field. Pinoxaden provides excellent control of yellow foxtail at recommended label rate. Peppers et al. (2020) found a similar result with an I_{50} of 3.4 g ai ha^{-1}. However, pinoxaden should not be considered for controlling knotroot foxtail even at the maximum recommended label rate. Sethoxydim provided effective control of yellow foxtail and reduced knotroot foxtail biomass by more than 60% at the labeled rate. Responses observed in this study suggest that differentiation between yellow and knotroot foxtail is essential before herbicide application.

Product used in this study

Anonymous (2011) Celsius ® WG product label. Carry, NC: Bayer Crop Science.

Anonymous (2008) Dismiss ® South, FMC Corporation, Philadelphia, PA.

Anonymous (2010) Dismiss® CA, FMC Corporation, Philadelphia, PA.

Anonymous (2020) Dupont® Steadfast, Corteva Agriscience, Wilmington, DE.

Anonymous (2018) Manuscript® product label, Syngenta Crop Protection, LLC Greensboro, NC.

Anonymous (2009) Scepter T&O, AMVAC Chemical Corporation, Newport Beach, CA.

Anonymous (2007) Sethoxydim TM; BASF Corporation, Research Triangle Park, NC.

Literature cited

Anonymous (2018) Manuscript® product label. Syngenta Crop Protection, LLC Greensboro, NC: .

Bryson CT, DeFelice MS (2009) Weeds of the south Athens, GA: University of Georgia Press. Pp. 495

Coats GE, Taylor JM, Kelly ST, Helms RB, Scott TD (1999) Highway vegetation management Mississippi: Miss. Agric. and Forest. 122 p

Darmency H, Dekker J (2011) Setaria. Pages 275-291 *in* Kole C, ed. Wild Crop Relatives : Genomic and Breeding Resources. Clemson: Springer

Dekker J (2003) The foxtail (Setaria) species-group. Weed Science 51:641-656

Dyer LM, Henry GM, McCullough PE, Belcher J, Basinger NT (2022) Knotroot Foxtail [Setaria parviflora (Poir.) Kerguélen]:"A sly fox". Weed Technology:1-22

Fry JD, Gaussoin RE, Beran DD, Masters RA (1997) Buffalograss Establishment with Preemergence Herbicides. Hortscience 34

Hadley W, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V T, akahashi K, Vaughan D WC, Woo K YH (2019) Welcome to the tidyverse. Journal of Open Source Software 4:1686

Hitchcock AS (1971) Manual of the grasses of the United States. New york: Dover

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. Weed Technology 21:840-848

Kotu V, Deshpande B (2018) Data science: concepts and practice: Morgan Kaufmann

Maloy BM (1985) Selective control of perennial grasses in Kentucky

blue-grass (Poa pratensis L.) turf with chlorsulfuron. Master of science Iowa Iowa State University

Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis

Peppers JM, Gonçalves CG, McElroy JS (2020) Rate response of select grass weeds to pinoxaden. Weed Technology 34:818-823

Ritz C, Baty F, Streibig J, Streibig C, Gerhar D (2015) Dose-Response Analysis Using R. PLOS ONE 10

Rominger JM (1962) Taxonomy of Setaria (Gramineae)

in North America *in* Press TUoI, ed. Urbana

RStudio T (2020) RStudio: Integrated Development Environment for R *in* RStudio I, ed. Boston, MA: R Foundation for Statistical Computing

Rusell D (2021) Widespread Foxtail Distribution Leads to Tough Management Decisions. Forages

Seefeldt SS, Jensen JE, Fuerst EP (2017) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218-227

Wang RL, Wendel JF, Dekker JH (1995) Weedy adaptation in Setaria spp II Genetic diversity and population genetic. American Journal of Botany 82:1031-1039

Table 1 :Yellow and knotroot foxtail control and above-ground biomass reduction relative to the non-treated (%ABRN) in response to herbicide treatments at 28 DAA

		Yellow foxtail		Knotroot foxtail	
Treatment	Rate	Control	%ABRN	Control	%ABRN
	g ai ha^{-1}	28 DAA			
Pinoxaden (low)	35	100 a	100 a	10 bc	14 cd
Pinoxaden (high)	70	87 a	96 a	20 c	30 bcd
Sethoxydim (low	316	100 a	100 a	40 abc	47 abcd
Sethoxydim (high)	520	100 a	100 a	77 a	94 ab
Thiencarbazone+dicamba+iodosulfuron	230	99 a	100 a	69 ab	90 ab
Metribuzin	395	100 a	99 a	81 a	96 a
Nicosulfuron+rimsufuron	563	98 a	98 a	64 abc	90 ab
Sulfentrazone	330	98 a	100 a	91 a	96 a
Sulfentrazone+Imazethapyr	504	93 a	100 a	62 abc	78 abc
Imazaquin	550	99 a	100 a	72 a	92 a

The values with the same letters have no significant difference. DAA : Days after application. Tukey HSD (*p=0.05)*. Non-treated controls were not included in the analysis due to all rates by zero for control.

Table 2: Yellow and knotroot foxtail control in field at 28 DAA

Treatment	Rate	Yellow foxtail		Knotroot foxtail	
		Year			
		2021 & 2022		2021	2022
	g ai ha^{-1}	28 DAA			
Pinoxaden (low)	35	53	abc	27 bc	13 abc
Pinoxaden (high)	70	67	ab	42 ab	15 ab
Sethoxydim (low	316	61	abc	23 bc	15 abc
Sethoxydim (high)	520	78	a	64 a	60 a
Thiencarbazone+dicamba+iodosulfuron	230	79	a	80 a	50 a
Metribuzin	395	50	abc	22 bc	25 abc
Nicosulfuron+rimsufuron	563	32	c	15 bc	33 c
Sulfentrazone	330	55	abc	25 bc	25 abc
Sulfentrazone+Imazethapyr	504	50	abc	17 bc	15 abc
Imazaquin	550	44	bc	27 bc	8 bc

The values with same letters have no significant difference. DAA : Days after application. Tukey HSD (*p=0.05)*. Non-treated controls were not included in the analysis due to all rates by zero for control.

Table 3 : Best fit model for percent visible injury and relative above ground biomass to the non-treated for yellow and knotroot foxtail in response to increasing rates of sethoxydim and Pinoxaden.

Herbicides	Species	[a]Equation	*I50 in g.ai/ha*	*Estimate I_{50} (95% CI) in g.ai/ha*
Pinoxaden	yellow foxtail	$f(x) = -19.82 + (119.82) \times (1 - \exp\{-\exp[0.45(\log(x) - \log(0.71))]\})$	6.45	[2.43; 17.92]
	knotroot foxtail	$f(x) = f(x) = -8.6 + (108.6) \times (1 - \exp\{-\exp[1.45(\log(x) - \log(2.36))]\})$	251.18	[128.7; 506]
Sethoxydim	yellow foxtail	$f(x) = 24.1 + (100 - 24.1) \times (1 - \exp\{-\exp[5.54(\log(x) - \log(2.13))]\})$	95.49	[58.83; 154.9]
	knotroot foxtail	$f(x) = -0.69 + (100 + 0.69) \times (1 - \exp\{-\exp[4.60(\log(x) - \log(3.59))]\})$	2089.29	[1380.38; 3235.90]

[a] *Regression parameter determined by Weibull model with four parameters for Pinoxaden data defined by equation 1 :*

Abbreviation: CI, confidence interval. I_{50}, Herbicide rate giving 50% control

Table 4 : Best fit model for percent biomass reduction of yellow and knotroot foxtail in response to increasing rates of sethoxydim and Pinoxaden

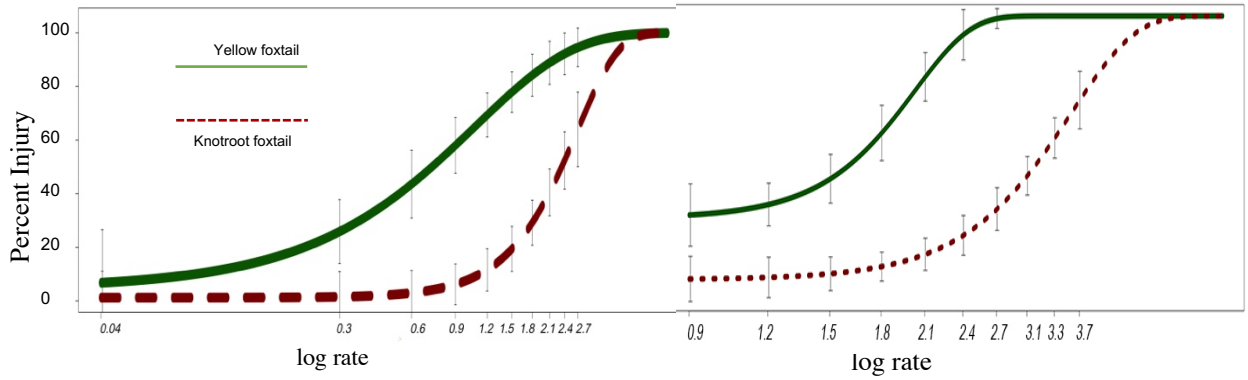
Herbicides	Species	Equation	*Log WR_{50}*	*WR_{50} in g.ai/ha*	*Estimate WR_{50} (95% CI) in g.ai/ha*
[a]Pinoxaden	yellow foxtail	$f(x) = \frac{100}{1 + [\exp - 1.64(logx - \log(0.20)]}$	0.20	1.62	[1.36; 1.91]
	knotroot foxtail	$f(x) = \frac{100}{1 + [\exp - 6.14(logx - \log(1.58)]}$	1.58	38.01	[30.45; 47.97]
[b]Sethoxydim	yellow foxtail	$f(x) = 100 \times (1 - \exp\{-\exp[-4.08(\log(x) - \log(1.18))]\})$	1.29	19.77	[13.73; 28.48]
	knotroot foxtail	$f(x) = 100 \times (1 - \exp\{-\exp[-18.30(\log(x) - \log(2.17))]\})$	2.21	165.72	[134.17; 204.702]

[a] Regression parameter determined by log-logistic with 4 parameters for pinoxaden data defined by equation 2

[b] Regression parameter determined by Weibull model with four parameters for sethoxydim data defined by equation 1:

abbreviation: CI: Confidence interval. WR_{50},: Herbicide rate giving 50% biomass reduction, respectively.

Figure 1. Percent visible injury to the nontreated control of yellow and knotroot foxtail 28 days after treatment with increasing rates of pinoxaden and sethoxydim.



Regression parameter determined by Weibull model with 4 parameters by equation 2: Left: Pinoxaden. Right: Sethoxydim

Each bar represents different herbicides and surrounds by their standard error. DAA: Days after application

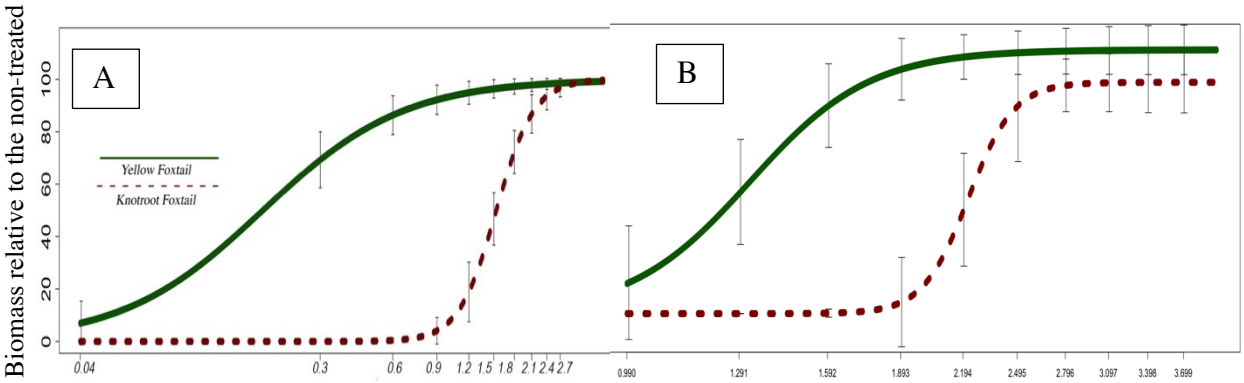


Figure 2. Biomass relative to the non-treated for yellow and knotroot foxtail 28 days after treatment with increasing rates of pinoxaden and sethoxydim.

[A]Regression parameter determined by log logistic with four parameters by equation 2: Left: Pinoxaden. Right: Sethoxydim

[B]Regression parameter determined by Weibull model with four parameters for sethoxydim data defined by equation 1:

Each bar represents different herbicides and surrounds by their standard error. DAA: Days after application

Chapter III.

Differentiation between yellow foxtail (*Setaria pumilla*) and knotroot foxtail (Setaria parviflora) via morphological characteristics and genetic markers

Abstract

Weeds are primarily identified through morphological characteristics. However, weed identification using morphological aspects requires botanical expertise, and subtle differences between species can vary with subspecies or biotypic morphological differences. *Setaria pumila* (Poir.) Roem. & Schult. (1817) and *Setaria parviflora* (Poir.) Kerguelen, respectively known as yellow and knotroot foxtail, are two problematic weed species that share morphological similarities and are often misidentified. Yellow and knotroot foxtail have phenotypic plasticity inter and intra-species, which can bias their identification. The criteria to differentiate those two species, such as the seedhead and rhizomes appears late in the growth stage, long after identification is required for herbicide management. Nucleic acids, DNA or RNA, are obvious options for identifying yellow and knotroot foxtail across biological systems beyond physical or digital identification. Research was conducted to differentiate yellow and knotroot foxtail beyond morphological characteristics using DNA barcoding. 22 biotypes of yellow foxtail, 25 biotypes of knotroot foxtail, and 7 foxtails classifieds as unknown were identified and collected in Alabama using seedhead size, ligule size, pubescence, and the presence of rhizomes. DNA for each biotype was isolated from the leaves and amplified using polymerase chain reaction (PCR) with primers *targeting trnH-psbA, rbcl, matK, and ITS* regions. Our result showed that knotroot foxtail is branched from the root base, which is usually purple and extends upward to eventually fades to green for the remainder of the leaf blade, while yellow foxtail is branched above the base. This is one of the key characteristics to differentiate the two species at early stages before the appearance of rhizome or seadhead in knotroot foxtail. Sanger sequencing results of the three-barcoding region, except *psbA*, demonstrate the presence of different single nucleotide polymorphisms in the sequenced regions that can differentiate yellow and knotroot foxtail. Some biotypes classified as unknown from their morphological characteristics were later correctly identified as yellow foxtail or knotroot foxtail by DNA barcoding. This study demonstrated that applying these DNA barcodes is relevant in accurately identifying foxtail species at an early stage and thus helps effectively manage them using proper herbicide selection.

Additional index words: Yellow, knotroot, foxtail, control, turfgrass,

Nomenclature: *Setaria pumila* (Poir.) Roem. & Schult. (1817), *Setaria parviflora* (Poir.) Kerguelen

Introduction

Weeds are undesirable due to their potential competition for resources against desirable plants and their contribution to crop economic loss (Oerke, 2005). Weeds plants can be controlled by applying physical, biological, and chemical measures, which is the most economically efficient (Oerke, 2005; Pimentel, 2005). Identification and correct herbicide selection constitute the cornerstone of efficient weed management programs; however, selective herbicides may result in limited or no control without proper identification (Naidu, 2012). The primary mechanism for identifying weeds is through morphological and botanical features; however, weed identification using physical characteristics requires botanical expertise, and subtle differences between species can vary with subspecies (Wang et al., 2017). Moreover, phenotypic plasticity and genetic variability can bias the identification of closely related species (Valentini et al., 2009). In addition, morphological criteria for differentiation are sometimes only effective for a particular life stage of the species (Valentini et al., 2009).

Currently, the development of computer vision and machine learning algorithms, such as deep learning, allow the identification of weeds, whereby we no longer need botanical expertise to identify plants (Hu et al., 2021; Pierre et al., 2018; Wu et al., 2021). However, computational identification requires large-quality samples, vegetative or reproductive materials, and plant parts that do not diverge significantly from the training data (Pierre et al., 2018). In addition, sample plant pictures only contain partial information, limiting the detection with high accuracy (Pierre et al., 2018).

The foxtails are a compelling example of this situation with phenotypic plasticity inter and intra-species. Yellow and knotroot foxtail, are two common species infesting turf in the southeastern region of the United States and share morphological traits (Bryson and DeFelice, 2009; Darmency and Dekker, 2011; Hitchcock, 1971). The criteria to differentiate those two species appear late in the growth stage, long after identification is required for herbicide management (Darmency and Dekker, 2011). Knotroot foxtail is one of the most widespread and morphologically diverse *Setaria* species native to the North American continent and can cross-hybridize with yellow foxtail, which originated to Eurasia (Doust and Diao, 2017; Rominger, 1962b). Previous research using the chloroplast gene ndhF and GISH experiments confirm the genomic similarities between yellow and knotroot foxtail (Dekker, 2003; Wang, 1994; Zhao et al., 2013b). Nevertheless, yellow and knotroot foxtail differ in their life cycle but share morphological

similarities (Dekker, 2003; Wang, 1994). The primary morphological differences between yellow and knotroot foxtail are found in the seedhead (Darmency and Dekker, 2011). Other studies specify that the rhizomes of knotroot foxtail are the only distinctive morphological characters (Wang et al., 1995). However, the morphological criteria to differentiate those species, such as the seedhead and rhizomes, appear late in the growth stage, long after identification is required for herbicide management.

Novel molecular technology has demonstrated great potential for rapid and reliable methods for plant identification (Group, 2009). Nucleic acids, DNA or RNA, are obvious options for the identification of species across biological systems beyond that of physical or digital identification (Hebert et al., 2003). The emergence of new sequencing techniques, such as Next-Generation sequencing (CBOL Plant Working Group et al.), ONT MinION nanopore sequencing, and Pac Bio sequencing allow the sequencing of nucleic acid at chloroplast and nucleus regions, which become an ideal framework for identifying closely related species (Antil et al., 2022; Kress and Erickson, 2008). DNA barcoding is a base genotyping method that relies on a standardized DNA region as a tag and is extensively applied in rapid and accurate species identification (CBOL Plant Working Group et al., 2009).

Different genes regions have been extensively used in plant DNA barcoding and the most common are *rbcL, matK, atpF-atpH, psbK-psbI, trnH-psbA,* and *ITS* (CBOL Plant Working Group et al., 2009; Hasebe et al., 1994; Lahaye et al., 2008). The gene *rbcl,* encoding the ribulose-bisphosphate carboxylase located in the chloroplast, is almost ubiquitous among plants and popular in phylogenetic inference due to its slow nucleotide substitution rate and its conservative mode of evolution (Hasebe et al., 1994; Judd et al., 1999; Palmer et al., 1988). Previous research has demonstrated that *rbcl* is the best-characterized gene for species discrimination due to its high universality and performance in high-quality bidirectional sequences(CBOL Plant Working Group et al., 2009; Clegg, 1993). However, the *rbcl* gene is limited since it provides low-resolution sequence compared to other barcode gene (Clegg, 1993). In contrast, the multicopy nuclear Internal Transcribed Spacer (Hasebe et al.) and the non-coding plastid trnH-psbA intergenic spacer are two alternatives to the *rbcl* gene (Kress et al., 2005). *TrnH-psbA* and *ITS* provide high-resolution sequences but are limited in sequence length, sequence alignment, or sequence

consistency, limiting the species discrimination (CBOL Plant Working Group et al., 2009; Kress et al., 2005; Zimmermann et al., 2013). However, the Consortium for the Barcode of Life (CBOL) proposed *matK+rbcL* to be the core plant barcode for species differentiation (CBOL Plant Working Group et al., 2009)

In this study, we evaluated the potential of the barcoding region *rbcl, ITS, trnH-psbA,* and *matK* to differentiate foxtail species in parallel to their morphological discrimination. We also perform an evolutionary analysis of yellow and knotroot foxtail and their classification using those primers' region.

Materials and methods

Sampling and morphological description: Biotypes of yellow and knotroot were collected in turfgrass, sidewalks, roadsides, and pastures in different regions in Alabama, with 22 biotypes of yellow foxtail, 25 knotroot foxtails, and 7 classifieds as unknown. USDA/ARS National Plant Germplasm System provided 4 other samples, which were yellow foxtail, knotroot foxtail, green foxtail, and giant foxtail. Plants collected were identified using seadhead size, ligule size, pubescence, or the presence of rhizomes. We also cleaned and analyzed two mature yellow and knotroot foxtail biotypes for botanical comparison. Relevant sections of each plant, such as ligule, leaf size, leaf hair, seedhead size, and roots system, were scrutinized and captured using Canon t5 camera EOS with different macro lenses and Meiji EMZ dissecting microscope. The identification key and plant descriptions presented are edited and modified from Diggs et al. (2006) , Rominger (2003), Weakley (2020), and helpful insights were gained from A.R. Diamond, Jr. (pers comm.) and D.D. Spaulding (pers comm.)

DNA extraction and barcoding: Freshly collected leaf tissue of approximately 100 mg was grounded using a bead mill homogenizer for each biotypes (Omni International, Kennesaw, GA). DNA was extracted using E.Z.N.A Plant kit (OMEGA-biotek, USA) following manufacturer instructions. DNA biotypes with nucleic acid concentrations inferior to 20 ng/ µl were excluded from the studies. In general, a PCR reaction was conducted using 20 ng of DNA template, 200 nM of the forward and reverse primers, 200 µM deoxyribonucleotide triphosphate (dNTPs), the 1X concentration of standard buffer, Taq polymerase (New England Biolabs Inc, Ipswich,

Massachusetts), and water to bring the final volume to 25 µL. The PCR product was examined on an agarose gel before the PCR cleanup step and sent sequencing to Eurofins, USA.

Sequence and evolutionary analysis by Maximum Likelihood method. Prior to the sequence analysis, sequences of each biotype were assembled, cut, and aligned using Bioedit. Unweighted Pair Group Method and the arithmetic mean were also used for sequence comparison and SNPs identification. Prior to the evolutionary analysis all the primer region for each biotype was combined, except for *trnH-psbA* regions. The evolutionary history was inferred using the Maximum Likelihood method and General Time Reversible model (Nei and Kumar, 2000). The bootstrap consensus tree was inferred from 1000 replicates and taken to represent the evolutionary history of the taxa-analyzed (Felsenstein, 1985). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6158)). The rate variation model allowed for some sites to be evolutionarily invariable. This analysis involved 48 nucleotide sequences. There were a total of 2728 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018)

Result and discussion

Morphological description. Yellow foxtail closely resembles knotroot foxtail and standard measurements of culm lengths, blade widths, panicle, and floret lengths often overlap between species making identification a challenge. No single character taken on its own is sufficient for distinguishing the two species--it takes evaluation of multiple characters at once. The presence or absence of a hardened rhizome is perhaps the best character for distinguishing the two species (fig. 4). Therefore, if roots are not collected and inspected closely along with other key characteristics one may not arrive at a satisfactory identification. However, at early stage, knotroot foxtail is branched from the root base, while yellow foxtail is branched above the base. (Fig. 1 and 2).
A second useful characteristic is spikelet length, and *S. parviflora* typically has smaller spikelets overall ranging from 1.8-2.8 mm (vs. 2.7-3.5 mm in *S. pumila*). Several character states are

similar between these species (e.g. both have glabrous sheaths and nodes) and many other measurements overlap, often leading to troublesome identifications. Overall, *S. parviflora* has shorter and more slender panicles from 1-8 cm (rarely to 10 cm), versus 3-12 cm (rarely to 15 cm) in *S. pumila*), and upper leaf blade surfaces are slightly scabrous with scarce to scattered papillose hairs at the leaf bases (vs. more abundant papillose-based hairs on leaves of *S. pumila*). The upper or fertile lemmas in *S. parviflora* are finely transversely rugose (cross-hatched patterned) compared with *S. pumila,* which has much more coarsely transversely rugose upper lemmas. In practice, plants of *S. parviflora* are generally smaller in many measurements when compared to individuals of *S. pumila*.

Sequencing analysis. The primer sequences of *ITS*, *matK*, *psbA*, and *rbcl* were correctly amplified in the different conserved regions of yellow and knotroot foxtail, with 80% successfully amplified and sequenced. We obtained a total of 192 sequences from 48 biotypes. The sequence size ranged from 740 to 753 base pairs (bp) for *rbcl*, 795 to 798 bp for *matK*, 734 to 738 for *ITS*, and 411 to 413 for psbA (Table 1). By comparing ITS region of the 4 samples from USDA germplasm, yellow and knotroot foxtail share 98% of similarities, but yellow foxtail showed 96% and 92% similarities to giant foxtail and green foxtail, respectively. Knotroot foxtail showed 95% and 97% similarities to giant foxtail and green foxtail, respectively. We observed similar results in rbcl and matK regions with higher similarities between yellow and knotroot foxtail than a single comparison of those to giant or green foxtail. No single nucleotide polymorphism (SNPs) was identified in pbsA region; however, ITS marker discriminated yellow and knotroot foxtail with 16 SNPs detected. Rbcl region performed less with 12 SNPs, followed by matK with 7 SNPS. However, in the rbcl region, we identified a consecutive base deletion in knotroot foxtail at 161 to 166 bp. However, this delete short sequence is not present in yellow foxtail, allowing us to differentiate the species. A total of 7 foxtail biotypes classified as unknown were correctly identified, as 4 to knotroot foxtail and 3 to yellow foxtail.

Evolutionary analysis by Maximum Likelihood method. Before the analysis, we concatenated the three-barcoding regions matK, ITS, and rbcl to form a sequence ranging from 2273 to 2728 bp. We identified no SNPs in psbA region, so we excluded psbA from the analysis. The Neighbor-Join trees constructed with 1000 bootstrapped replication showed that the 48 biotypes grouped into three main clusters, with giant and green foxtail forming two different clades Yellow and knotroot foxtail were in the same group, forming a specific monophyletic clade with a bootstrap value equal to 99, based on the genetic difference. All the biotypes morphologically identified as yellow foxtail and knotroot foxtail were clustered in the same sub-clade with bootstrap values from 9 to 15. The unknown biotypes were clustered as 4 to knotroot foxtails and 3 to yellow foxtails (fig. 7).

Discussion. The complexity and inaccuracy in classification due to the high degree of overlapping morphological characters within and between species pose a challenge for species identification in the genus *Setaria* (Dekker, 2003; Dekker, 2004). Dyer et al. (2023) described knotroot foxtail and found that the presence of rhizomes and purple coloration present above the crown as key characteristics of differentiating knotroot from others closely *Setaria*. Early studies have specified that the species are differentiated by their life cycle (yellow foxtail: annual, knotroot foxtail : perennial) or by the spikelet length (2 to 2.5 mm for knotroot foxtail compared with 3 mm for yellow foxtail) (Hitchcock, 1971; Hitchcock and Chase, 1951). From our analysis, knotroot foxtail branching from the base is a key characteristic to differentiate it from yellow foxtail at early stages (fig 1). Another difference at the early stage is that yellow foxtail is pubescence with uniform leaf hairs along the entire leaves, while knotroot foxtail is only pubescent at the base of the leaf blades, near the ligules (fig 3). However, those differences are subtly leading to potential misidentification in the field.

In this study, we test highly conserved universal primers to obtain ideal DNA barcoding sequences to differentiate foxtail species. *MatK* and *rbcl* are the core loci recommended by CBOL for plant DNA barcoding (CBOL Plant Working Group et al., 2009). However, Singh et al. (2016) showed that *rbcl* genes could not distinguish all the *Setaria* species because *rbcl* is highly conserved at intragenic level (Singh et al., 2016). Syme et al. (2013) tested the barcoding loci *rbcL, matK* and *ITS* in several grass species and found that rbcl was the best to discriminate stipoid grasses. However, in this study, we found that *rbcl* can discriminate the four foxtail species. All the collected biotypes match the USDA germplasm biotypes, either for yellow or knotroot foxtail.

Wang et al. (2017) evaluated the potential of matK to differentiate grass species and found that matK provided weak support for differentiating some close grass species. However, we identified SNPs in this region, allowing foxtail discrimination. Zimmermann et al. (2013) found that a combination of matK and rbcl have a great discrimination power in the genus panicum, which includes *Setaria*. However, It has been suggested that the combination of matK and rbcl are not powerful enough to distinguish closely related species (Spooner, 2009; Starr et al., 2009). The combination of ITS, matK, and rbcl in this study correctly clustered the foxtail biotypes using the Neighbor-Join analysis. We observed no SNPs in PsbA, similar to previous studies that demonstrated the Trnh-PBSA region posed difficulties in amplification, sequencing alignment, and species discrimination (Spooner, 2009; Zhang et al., 2012). Spooner (2009) found that Trnh-PBSA sequences are parsimony informative in some closely related solanum species with nucleotide sometimes interspersed with a 1-bp insert of another nucleotide, which complicated the obtention of good sequence from direct sequencing. We find a similar pattern in yellow and knotroot foxtail with no SNPs identified and with overlaid bootstrap values and incorrect clade in psbA.

Conclusion

DNA barcoding provides an important tool for species identification beyond morphological characteristics. *ITS, rbcl, and matK* primers are the primary candidate for amplified conserved regions to further discriminate yellow and knotroot foxtail. This study compared the accuracy of 48 foxtail sample sequences to USDA sample using the three barcoding regions and revealed 100% correct identification. The absence of the reference genome of yellow and knotroot foxtail limited this study to perform a BLAST which will allow testing the accuracy of our sequence. Since foxtails show significant variability intra-species and populations, samples outside the southern region of the United States must be tested to support the barcoding accuracy.

Literature cited

Anonymous (2018) Manuscript® product label. Syngenta Crop Protection, LLC Greensboro, NC: .

Antil S, Abraham JS, Sripoorna S, Maurya S, Dagar J, Makhija S, Bhagat P, Gupta R, Sood U, Lal R, Toteja R (2022) DNA barcoding, an effective tool for species identification: a review. Mol Biol Rep

Austin DF (2006) Illustrated Flora of East Texas: Volume 1. Introduction, Pteridophytes, Gymnosperms, and Monocotyledons. Economic Botany 60:397-398

Beard JB, Green RL (1994) The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans. Journal of environmental quality 23:452-460

Bhowmik PC (2017) Weed biology: importance to weed management. Weed Science 45:349-356

Bhowmik PC, Bingham SW (2017) Preemergence Activity of Dinitroaniline herbicides Used for Weed Control in Cool-Season Turfgrasses. Weed Technology 4:387-393

Brosnan JT, Elmore MT, Bagavathiannan MV (2020) Herbicide-resistant weeds in turfgrass: current status and emerging threats. Weed Technology 34:424-430

Bryson CT, DeFelice MS (2009) Weeds of the south Athens, GA: University of Georgia Press. Pp. 495

CBOL Plant Working Group, Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, Chase MW, Cowan RS, Erickson DL (2009) A DNA barcode for land plants. Proceedings of the National Academy of Sciences 106:12794-12797

Chikara J, Gupta PK (1980) Numerical taxonomy in the genus Setaria (L.) Beauv. Indian academic Plant Science 89:401-406

Clegg MT (1993) Chloroplast gene sequences and the study of plant evolution. Proceedings of the National Academy of Sciences 90:363-367

Coats GE, Taylor JM, Kelly ST, Helms RB, Scott TD (1999) Highway vegetation management Mississippi: Miss. Agric. and Forest. 122 p

Darmency H, Dekker J (2011) Setaria. Pages 275-291 *in* Kole C, ed. Wild Crop Relatives : Genomic and Breeding Resources. Clemson: Springer

Dekker J (2003) The foxtail (Setaria) species-group. Weed science 51:641-656

Dekker J (2004) Evolutionary Biology of the Foxtail (Setaria) Secies-Group. Weed biology and management:65-113

Doust A, Diao X (2017) Genetics and genomics of Setaria: Springer

Dyer LM, Henry GM, McCullough PE, Belcher J, Basinger NT (2022) Knotroot Foxtail [Setaria parviflora (Poir.) Kerguélen]:"A sly fox". Weed Technology:1-22

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791

Flessner M, Rector L, Pittman K (2018) Control of common grassy weeds
in pastures and hayfields. Virginia Virginia Tech

Fry JD, Gaussoin RE, Beran DD, Masters RA (1997) Buffalograss Establishment with Preemergence Herbicides. Hortscience 34

Group CPW (2009) A DNA barcode for land plants. Proc Natl Acad Sci U S A 106:12794-7

Hadley W, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V T, akahashi K, Vaughan D WC, Woo K YH (2019) Welcome to the tidyverse. Journal of Open Source Software 4:1686

Hasebe M, Omori T, Nakazawa M, Sano T, Kato M, Iwatsuki K (1994) rbcL gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proceedings of the National Academy of Sciences 91:5730-5734

Hebert PD, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc Biol Sci 270:313-21

Hitchcock AS (1971) Manual of the grasses of the United States. New york: Dover

Hitchcock AS, Chase A (1951) Manual of the grasses of the United States. 2nd / rev. by Agnes Chase. edn. Washington, D.C: U.S. Dept. of Agriculture

Hu K, Wang Z, Coleman G, Bender A, Yao T, Zeng S, Song D, Schumann A, Walsh M (2021) Deep Learning Techniques for In-Crop Weed Identification: A Review. arXiv

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ (1999) Plant systematics: a phylogenetic approach. Ecología mediterránea 25:215

Kellogg Elizabeth A (2017) Evolution of Setaria *in* Doust AN, Diao X, eds. Genetics_and_Genomics_of_Setaria. Switzerland: Springer

Kellogg Elizabeth A, Aliscioni Sandra S, Morrone O, Pensiero J, Zuloaga F (2009) A Phylogeny ofSetaria (Poaceae, Panicoideae, Paniceae) and Related Genera Based on the Chloroplast GenendhF. International Journal of Plant Sciences 170:117-131

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. Weed Technology 21:840-848

Kotu V, Deshpande B (2018) Data science: concepts and practice: Morgan Kaufmann

Kress WJ, Erickson DL (2008) DNA barcodes: genes, genomics, and bioinformatics. Proc Natl Acad Sci U S A 105:2761-2

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences 102:8369-8374

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35:1547-1549

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, Gigot G, Maurin O, Duthoit S, Barraclough TG, Savolainen V (2008) DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci U S A 105:2923-8

Maloy BM (1985) Selective control of perennial grasses in Kentucky

blue-grass (Poa pratensis L.) turf with chlorsulfuron. Master of science Iowa Iowa State University

Manthey DR, Nalewaja JD (1987) Germination of Two Foxtail (Setaria) Species. Weed Technology 1:302-304

Milton J-H (1998) Characterization of foxtail (Setaria spp.) seed production and

giant foxtail (S. faberi) seed dormancy at abscission. Doctor of Philosophy Ames: Iowa State University

Mollard FP, Insausti P (2009) Breaking Setaria parviflora seed dormancy by nitrates and light is part of a mechanism that detects a drawdown period after flooding. Aquatic Botany 91:57-60

Montiel L, Mayra B (1975) Comparación anatómico-taxonómica de Setaria magna Griseb.

y Setaria geniculata (Lam .) Beauv. de Florida, EE. UU. Biologia tropical 24:95-197

Morrone O, Aliscioni Sandra S, Fritz VJ, Pensiero J, Zuloaga F, Kellogg Elizabeth A (2014) A revision of the Old World species of Setaria..pdf. Systematic botany monograph 93-95

Naidu VSGR (2012) Hand Book on Weed Identification. Jabalpur: Directorate of Weed Science Research,

Nei M, Kumar S (2000) Molecular evolution and phylogenetics: Oxford University Press, USA

Norris RF, Schoner CA (2017) Yellow Foxtail (Setaria lutescens) Biotype Studies: Dormancy and Germination. Weed Science 28:159-163

Oerke EC (2005) Crop losses to pests. The Journal of Agricultural Science 144:31-43

Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis

Palmer JD, Jansen RK, Michaels HJ, Chase MW, Manhart JR (1988) Chloroplast DNA Variation and Plant Phylogeny. Annals of the Missouri Botanical Garden 75:1180-1206

Peppers JM, Gonçalves CG, McElroy JS (2020) Rate response of select grass weeds to pinoxaden. Weed Technology 34:818-823

Pierre B, Hervé G, Siang TH, Mario L, Milaň S, Valéry M, Philippe J, Jean-Claude M, Christian Y, Alexis J (2018) Plant identification: Experts vs. machines in the Era of deep Learning. Pages 121-149 Multimedia tools and applications for environmental & biodiversity informatics: Multimedia Systems and Applications Series

Pimentel D (2005) 'Environmental and Economic Costs of the Application of Pesticides Primarily in the United States'. Environment, Development and Sustainability 7:229-252

Ritz C, Baty F, Streibig J, Streibig C, Gerhar D (2015) Dose-Response Analysis Using R. PLOS ONE 10

Rominger JM (1959) taxonomy and systematic of setaria. Doctor of Philosophy PhD dissertation Illinois: University of illinois 250 p

Rominger JM (1962a) Taxonomy of Setaria (Gramineae)

in North America *in* Press TUoI, ed. Urbana

Rominger JM (1962b) Taxonomy of Setaria (Gramineae) in North America: University of Illinois at Urbana-Champaign

RStudio T (2020) RStudio: Integrated Development Environment for R *in* RStudio I, ed. Boston, MA: R Foundation for Statistical Computing

Rusell D (2021) Widespread Foxtail Distribution Leads to Tough Management Decisions. Forages

Santelmann PW, Meade JA, Peters RA (1963) Growth and developement yellow foxtail and giant. Cambridge University Press 11:139-142

Schröder S, Bahri BA, Eudy DM, Layton DJ, Kellogg EA, Devos KM (2016) Genetic diversity and origin of North American green foxtail [Setaria viridis (L.) Beauv.] accessions. Genetic Resources and Crop Evolution 64:367-378

Seefeldt SS, Jensen JE, Fuerst EP (2017) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218-227

Singh A, Drishti NG, Mohanty A In silico analysis of sequence variation in rbcL gene to assess phylogenetic relations in Setaria species

Spooner DM (2009) DNA barcoding will frequently fail in complicated groups: an example in wild potatoes. American journal of botany 96:1177-1189

Starr JR, Naczi RF, Chouinard BN (2009) Plant DNA barcodes and species resolution in sedges (Carex, Cyperaceae). Molecular ecology resources 9:151-163

Steel MG, Cavers. PB, Lee SM (1983) The biology of canadian weeds. Setaria glauca (L.) Beauv. and S. verticillata (L.) Beauv. Canadian journal of plant science 711-725

Valentini A, Pompanon F, Taberlet P (2009) DNA barcoding for ecologists. Trends Ecol Evol 24:110-7

Walsh B, S. IS, J. B (1999) Biology and Management of Dollar Spot (Sclerotinia homoeocarpa); an Important Disease of Turfgrass. Hortscience 34:13

Wang A, Gopurenko D, Wu H, Lepschi B (2017) Evaluation of six candidate DNA barcode loci for identification of five important invasive grasses in eastern Australia. PLoS One 12:e0175338

Wang R-L (1994) weedy adaptation in setaria spp: Genetic diversity, population genetic structure and variation in herbicide resistance. Doctor of Philosophy. Iowa: Iowa State Univeristy. 124 p

Wang RL, Wendel JF, Dekker JH (1995) Weedy adaptation in Setaria spp II Genetic diversity and population genetic. American Journal of Botany 82:1031-1039

Weakley AS (2020) Flora of the southeastern United States: University of North Carolina at Chapel Hill Herbarium (NCU)

Wu Z, Chen Y, Zhao B, Kang X, Ding Y (2021) Review of Weed Detection Methods Based on Computer Vision. Sensors (Basel) 21

Yi J, Duffy PA (2012) Economic Analysis of Turfgrass-Sod Production in Alabama. Pages 1-92

Zabihollahi V, Meighani F, Karaminejad M, Mirhadi M (2008) Study of Chemical Control of Yellow Foxtail (Setaria glauca (L.) Beauv.) and Common Dandelion (Taraxacum syriacum boiss.) in Tall Fescue (Festuta arundinacea Schreb.) Turf. Iranian Journal of Weed Science 4:1-13

Zhang Cy, Wang fy, Yan hf, Hao g, Hu cm, Ge xj (2012) Testing DNA barcoding in closely related groups of Lysimachia L.(Myrsinaceae). Molecular Ecology Resources 12:98-108

Zhao M, Zhi H, Doust AN, Li W, Wang Y, Li H, Diao X (2013a) Novel genomes and genome constitutions identified by GISH and 5S rDNA and knotted1 genomic sequences in the genus Setaria. BNC Genomic

Zhao M, Zhi H, Doust AN, Li W, Wang Y, Li H, Jia G, Wang Y, Zhang N, Diao X (2013b) Novel genomes and genome constitutions identified by GISH and 5S rDNA and knotted1 genomic sequences in the genus Setaria. BMC genomics 14:1-12

Zimmermann T, Bocksberger G, Bruggemann W, Berberich T (2013) Phylogenetic relationship and molecular taxonomy of African grasses of the genus Panicum inferred from four chloroplast DNA-barcodes and nuclear gene sequences. J Plant Res 126:363-71

Figure 1**:** Knotroot foxtail (left) with purple coloration above the crown and branching from the base. Yellow foxtail (right) at early stage

Figure 2 : Similar Root system of knotroot foxtail (left) and yellow foxtail (right) at *early stage*

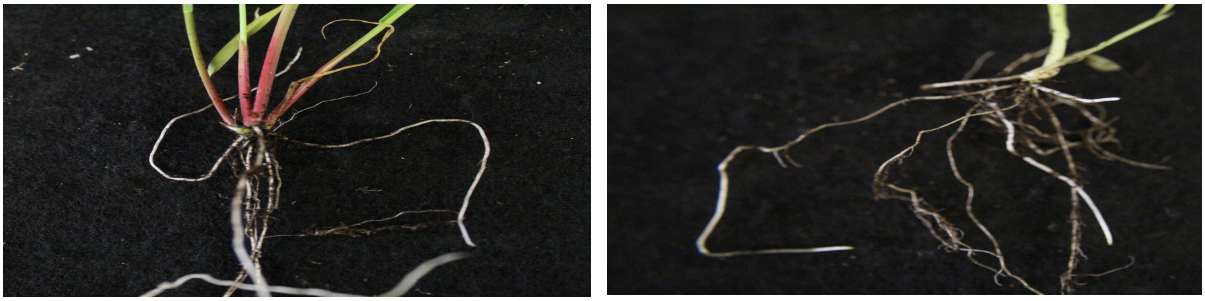


Figure 3 : Knotroot foxtail (left) with geniculate stem and yellow foxtail (right) at mature stage

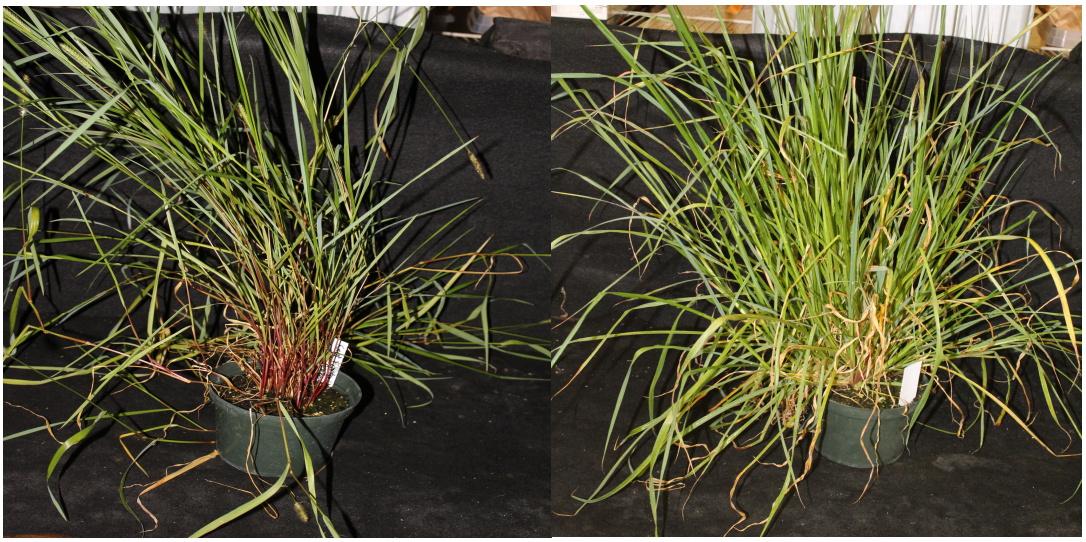


Figure 4 : Highlight the presence of rhizome in knotroot foxtail (left) and fibrous root yellow foxtail (right)

Figure 5 : Seedhead of knotroot foxtail (left) and yellow foxtail (right) bigger and denser

Figure 6 : Fig 6: Knotroot foxtail (left) presents with leaf hairs only near ligule, while yellow foxtail (right) presents longer leaf hairs along the entire leaf.

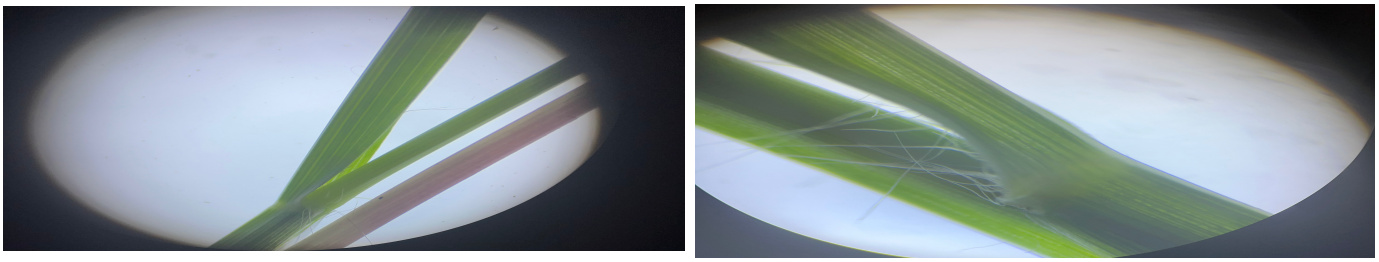


Table 5: GC content, base pair length(bp) and SNPs identified for yellow, knotroot foxtail, knotroot foxtail.

DNA barcoding region	**Forward primer sequence**	**Reverse primer sequence**	**GC content**	**BP length**	**SNPs identified**
ITS	ACGAATTCAAGGTCCGGTGAAGTGTTCG	TAGAATTCCCCGGTTCGCTCGCCGTTAC	410	734 to 738	16
rbcl	AGTAGTAGGATTGATTCTCA	CAACACTTGCTTTAGTCTCT	224	740 to 753	12
matK	CTATATCCACTTATCTTTCAGGAGT	AAAGTTCTAGCACAAGAAAGTCG	259	795 to 798	7
Trnh-psbA	GTTATGCATGAACGTAATGCTC	CGCGCATGGTGGATTCACAATCC	152	411 to 413	0

Figure 7: Phylogenetic tree developed using Maximum likelihood method, Neighbor-Joining, and visualized using Interactive Tree Of Life (iTOL). KF: knotroot foxtail. YF: Yellow foxtail UF : Unknown foxtail.GF : Giant foxtail. NF: Green Foxtail

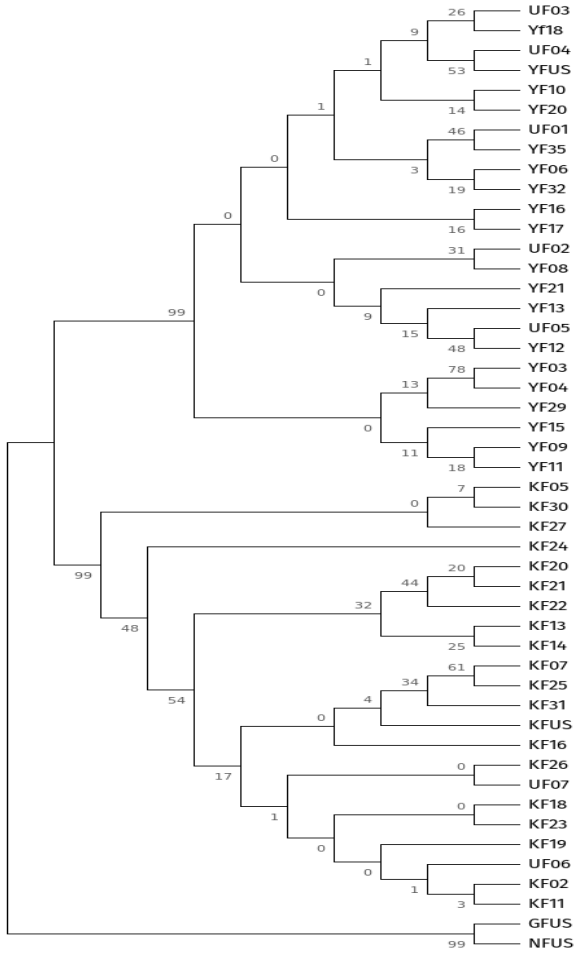


Figure 8: Map showing the distribution of the biotypes collected in Alabama, USA. Map realized in arcgis software 10.8.2

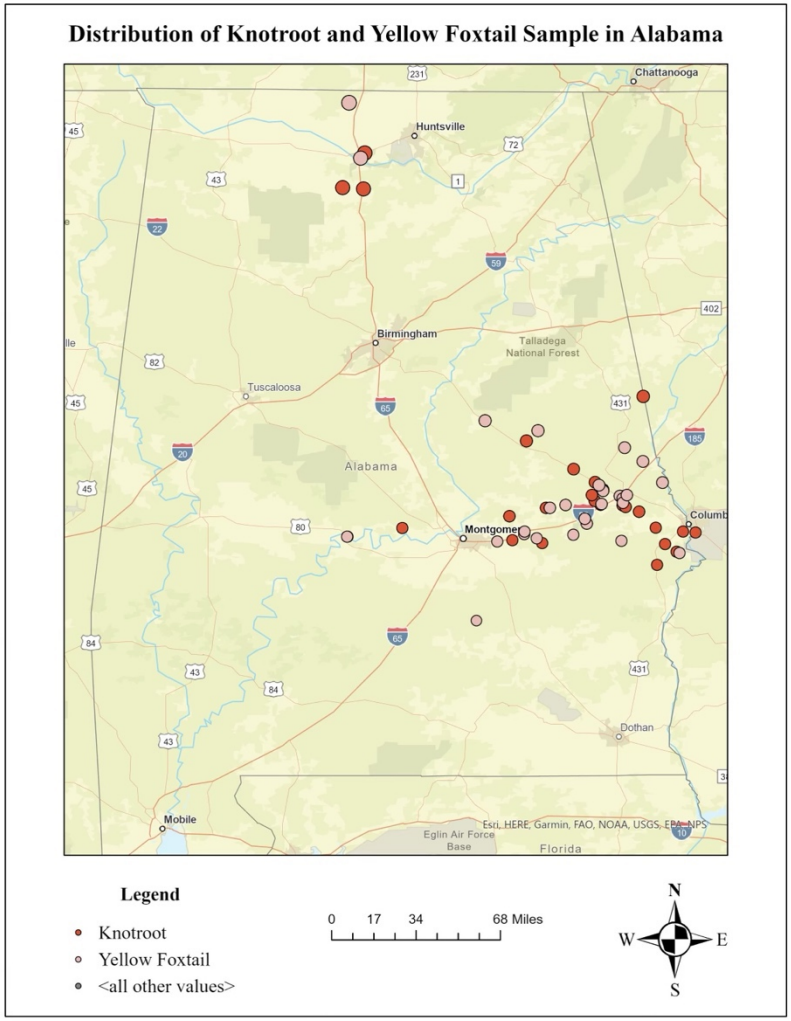


Figure 9 : Overview of matK sequence with different SNPs identified.