Biology and Chemical Control Strategies for Managing Knotroot Foxtail [*Setaria parviflora* (Poir.)] in Forage Systems

by

Tunde Lanre Akanbi

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 10, 2025

Keywords: Forage, Foxtail, Herbicide, Perennial, Rainfall, Rhizome

Approved by

Dr. David P. Russell, Chair, Assistant Extension Professor, Department of Crop and Environmental Sciences
Dr. Scott J. McElroy, Co-chair, Professor, Department of Crop and Environmental Sciences
Dr. Aniruddha Maity, Assistant Professor, Department of Crop and Environmental Science

Abstract

Knotroot foxtail is a perennial grass weed that reproduces by both seeds and rhizomes, making management difficult, as new shoots can emerge from underground rhizomes. There is a knowledge gap that exists on the time it takes knotroot foxtail to develop a rhizome following seedling emergence. Additionally, its close resemblance to yellow foxtail complicates identification and hinders effective management. A greenhouse and field study were conducted to investigate the developmental stages of knotroot foxtail from seed to rhizome and to distinguish it morphologically from yellow foxtail. Results showed that 50% of the knotroot foxtail population emerged by 8 days after sowing, while 95% of the population emerged by 14 days. These emerged seedlings transitioned into rhizome formation between 7 and 13 weeks after planting. At the Beef Unit, 50% of the knotroot foxtail population developed rhizomes at plant heights of 12.7–24.1 cm and the 1–2 leaf stage, while at the Turf Unit, the populations had formed at 40.6–52.6 cm and the 3–5 leaf stage. The average rhizome-root biomass when rhizome formation occurred among 95% of the knotroot foxtail plant population was 37.52 - 92.19 g. Time to seedhead formation among the knotroot foxtail population occurred between 59.8 and 66.8 days after planting.

For the morphological differentiation study between knotroot and yellow foxtail, Principal Component Analysis (PCA) identified quantitative traits such as first true leaf width, spikelet width, spikelet length, internode length, as distinguishing features between knotroot foxtail and yellow foxtail. In addition, qualitative traits including leaf margin roughness, leaf color, and seed size also differed between the two species. However, a single morphological trait alone might not suffice for identification as there might be need to combine these traits for effective identification especially when plants are young.

Hexazinone and quinclorac are two herbicide active ingredients shown to have efficacy on controlling knotroot foxtail, but their performance is influenced by soil moisture and rainfall activation. Understanding how soon rainfall is needed after herbicide application of these herbicides is essential for maximizing control and to manage for desirable forage species such as bermudagrass, bahiagrass, and tall fescue. Greenhouse studies in 2023 and 2024 at Auburn University evaluated knotroot foxtail response of knotroot foxtail to these herbicides under varied rainfall timings. Knotroot foxtail rhizomes were transplanted into pots and allowed to grow until foliage reached an average height of 28cm before being treated with 0.42 kg ae ha^{-1} quinclorac and 0.85 kg ai ha^{-1} hexazinone. Simulated rainfall of 0.63 cm (0.25 inches) was applied at 0, 3, 6, 9, 12, and 15 days after herbicide treatment (DAT). Hexazinone consistently reduced rhizome dry weight more than quinclorac across both years. In 2023, rhizome weight was 1.88 g in hexazinone-treated plants compared to 3.24 g with quinclorac. In 2024, knotroot foxtail treated with hexazinone had lower rhizome dry weight (1.02 g), followed by quinclorac at 2.09 g, while the non-treated control had the highest rhizome dry weight (3.58 g).

Similarly, across both years, hexazinone consistently caused higher knotroot foxtail control compared to quinclorac, with control levels of 93% and 82% at 51 days after rainfall treatment (DART) in 2023, and 94% and 79% at 51 DART in 2024, respectively. At 51 days after rainfall treatment (DART), knotroot foxtail plants treated with quinclorac exhibited control levels ranging from 87% to 77% when rainfall occurred within 0–9 days after application. However, control declined to between 67% and 62% when rainfall was delayed until 12–15 days after treatment. Early rainfall (0–6 days) ensured optimal herbicide efficacy, while late rainfall (12–15 days) reduced their effectiveness. This study provides insight into the biology of knotroot and yellow

foxtail and highlights how to optimize the efficacy of the limited herbicide options available for knotroot foxtail control in pastures and hayfields.

Acknowledgments

My sincere appreciation belongs to the Almighty God, the author and finisher of my faith who in his infinite mercies guided me throughout this study. I express my heartfelt gratitude to Dr. David Pierce Rusell for his excellent supervision and guidance throughout the course of this work, I benefited so much from your experience. Words cannot express my profound gratitude to you but will only pray that your seeds shall find God's favour in everything they lay their hands on, and I shall never forget your kindness towards me. I am also grateful for the rarest opportunity given to me by Dr. Scott McElroy and Dr. Aniruddha Maity to work and learn under their tutelage and supervision. My appreciation extends to the Head of Department (Dr. Steve Hague) and other members of staff (academic and non-academic) of Crop Soil and Environmental Science, Auburn University for their valuable and immense contribution to making me who I am today.

I am equally indebted to my parents, Late Mr. J. R. Akanbi and Mrs. E. Y. Akanbi for the spiritual, moral and emotional support they gave to me in ensuring the completion of the program. I pray that you will enjoy the fruits of your labor. My gratitude also goes to my siblings, Olatunde Peter, Akanbi David, Akanbi Segun, you are the best I have ever encountered in life and thanks for your encouraging words all through this program.

Finally, I extend my sincere gratitude to Forrest Davis, Debra, Will, LeAnn, and all my other lab mates whose names I cannot list here due to space. Your support throughout my time at Auburn University has been invaluable. Whenever I encountered challenges, you were always there to help me find solutions and keep moving forward. Each of you played a unique role in the successful completion of this research. Thank you.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

PCA	Principal Component Analysis
PC	Principal Component
RF	Rhizome Formation
HSD	Honest Significant Difference
LRT	Likelihood Ratio Test
RMSE	Root Mean Square Error
DART	Days after Rainfall Treatment
DAT	Days after Treatment
ANOVA	Analysis of Variance

Chapter I

Literature Review

Nomenclature of *Setaria* species

Setaria is a genus of monocotyledonous grasses in the Poaceae family, commonly referred to as foxtail or bristle grasses due to their distinctive cylindrical, bristly inflorescences that resemble a fox's tail (Webster, 1993). The genus comprises over 140 species found across tropical and temperate regions worldwide (Barkworth *et al*., 2007). The name *Setaria* is derived from the Latin word seta, meaning "bristle," and the suffix -aria, meaning "possessing," in reference to the prominent bristly inflorescence characteristic of species within this genus (Smith, 1860; Zimdahl,1989; Rominger, 1962; Barkworth *et al*., 2007). The Setaria genus includes both cultivated and weedy species, with several weedy types being particularly significant in North America (Dekker, 2003). Notable among these are *Setaria parviflora* (knotroot foxtail), *Setaria pumila* (yellow foxtail), *Setaria viridis* (green foxtail), and *Setaria faberi* (giant foxtail).

These species are economically important in weed science due to their competitive growth, prolific seed production, and increasing cases of herbicide resistance in various cropping systems (Holm *et al*., 1977; Wang and Dekker, 1994; Prasada Rao *et al*. 1987). Many of these grasses have undergone multiple nomenclatural revisions due to morphological similarities and evolving taxonomic interpretations (Rominger, 1962; Aliscioni *et al*., 2016). *Setaria pumila* (Poir.) Roem. and Schult., commonly known as yellow foxtail, was originally described as *Panicum pumilum* by Jean Louis Marie Poiret, a French botanist active in the late 18th century (Roemer and Schultes, 1817). The species was later reassigned to the Setaria genus by Johann Jacob Roemer and Josef August Schultes in 1817, whose names are retained in the current botanical citation (Flora of North

America, 2007). The epithet pumila, meaning “dwarf,” likely referred to the stature of the type specimen rather than reflecting the typical height of the species within the genus (Bhat *et al*., 2018). Over time, it has also appeared under synonyms such as *Setaria helvola*, *Setaria glauca*, and *Setaria lutescens* (Rominger, 1962). Common names like yellow bristlegrass, pigeon grass, and cattail grass have also been elucidated in the literature for yellow foxtail (Barkworth *et al*., 2007; Flora of North America, 2007; NRCS, 2016; Poiret, 1817).

Setaria parviflora (Poir.) Kerguélen, commonly called knotroot foxtail or knotroot bristlegrass, is a perennial species that propagates via rhizomes (Bryson and DeFelice, 2009). It was originally described as *Panicum parviflorum* by Poiret and reclassified under Setaria in 1987 (Kerguélen, 1987; Poiret, 1804). The specific name parviflora, meaning "small-flowered," describes its fine inflorescence. Synonyms such as *Cenchrus parviflorus* and *Setaria geniculata* have contributed to historical confusion in its taxonomy (Barkworth *et al*., 2007; Flora of North America, 2007; Kerguélen, 1987). The naming history of *Setaria parviflora* has gone through several changes. For a long time, the plant was known as *Setaria geniculata* P. Beauv. (1812) (Hitchcock, 1931, 1951). However, later work showed that Beauvois had actually referred to a different species *Panicum geniculatum*, published by Willdenow in 1809 (Willdenow, 1809). Since Willdenow’s name came after Lamarck’s and used the same epithet, it was considered invalid. To resolve this, Kerguélen in 1977 proposed using the oldest valid name available *Cenchrus parviflorus* Poir. and renamed the plant *Setaria parviflora* (Poir.) Kerguélen (Kerguelen, 1977). Although *S. geniculata* was a familiar name, it could not be retained because the nomenclatural rules at the time did not allow conservation of names unless the plant was economically important (Voss, 1983; Greuter, 1988). As a result, *Setaria parviflora* is now the accepted name (Webster, 1987; Pohl, 1962).

Origin and distribution of knotroot foxtail and yellow foxtail

Yellow foxtail (*Setaria pumila*) and knotroot foxtail (*Setaria parviflora*) are two distinct species within the Setaria genus that differ in origin, life cycle, and geographic distribution (Rominger, 1959). Both species are widespread weedy grasses in the United States and are known to cause significant yield losses in forage production systems (Dekker, 2003). Despite some morphological similarities, they have distinct evolutionary histories and biogeographic origins (Dekker, 2003). Yellow foxtail is generally considered to have originated in Eurasia (Rominger, 1959), though some phylogenetic analyses suggest it may be native to Africa, placing it closer to African Setaria species (Kellogg *et al*., 2009). Its global spread has been attributed to both natural dispersion and human-mediated activities (Stapf and Hubbard, 1930).

The invasion of yellow foxtail into the Americas came primarily from Asia, and its post-Columbian dispersal has largely been driven by Eurasian human migration over the past 500 years (Wang *et al*., 1995; Dekker, 2003). However, a prehistoric introduction to the New World cannot be ruled out, as some Setaria species were already present in the Americas prior to European contact (Dekker, 2003). The species is now widely distributed across the United States, South America (notably Argentina and Uruguay), Africa, Europe, Asia, Australia, and various Pacific islands, thriving in disturbed environments, pastures, roadsides, and agricultural fields.

Knotroot foxtail, in contrast, is a rhizomatous perennial believed to have originated in the Americas. It is the most widespread indigenous Setaria species in the Western Hemisphere (Rominger, 1959), with some evidence suggesting it may have spread northward from South America (Dekker, 2003). Remarkably, a species of Setaria likely knotroot foxtail was the oldest cultivated cereal in the Americas, dating back nearly 9,000 years before present (Callen 1965, 1967; de Wet 1992; Smith 1968). The prehistoric migration of Setaria may have coincided with

the movement of ancestral Native American populations across the Bering land bridge (Beringia) between 10,000 and 20,000 years ago (Dekker, 2003).

Today, knotroot foxtail occurs throughout the southern and eastern United States, parts of Central and South America, southern Africa, Europe, Asia, Australia, and several Pacific islands (Hitchcock, 1971). In the U.S., it is especially common along the Atlantic and Gulf coasts, from Massachusetts to Florida and westward to Texas, Kansas, and California (Hitchcock, 1971). Although yellow foxtail is an annual and knotroot foxtail is a perennial, both species share several morphological traits, suggesting a possible common ancestry. Wang (1994) proposed that they may have diverged relatively recently from a shared African ancestor. Their biogeographic patterns and potential gene flow remain poorly understood, underscoring the need for further phylogenetic and population genetic studies (Darmency and Dekker, 2011, Schröder *et al*., 2016).

Seed Biology and Life Cycle of Yellow Foxtail and Knotroot Foxtail

Yellow foxtail is an annual summer grass that reproduces solely through seeds. Its seeds typically exhibit primary dormancy at maturity, which gradually breaks down over time, allowing germination in early spring (Schoner *et al*., 1978). Germination is favored by warm soil temperatures, with optimal germination observed between 20°C and 30°C, and a base temperature of approximately 10.2°C (Norris and Schooner, 1980; Manthey and Nalewaja, 1987). Seeds of yellow foxtail can germinate from depths ranging between 2 mm and 50 mm, although emergence is highest when seeds are located in the top 2–15 mm of soil (Dawson and Bruns 1962). Seed longevity varies depending on environmental conditions, with some seeds remaining viable for 3–5 years, especially in undisturbed or no-till systems (Darlington, 1951, Kivilaan and Bandurski 1973). The persistence of its seedbank poses a significant challenge to weed control in annual cropping systems. Therefore, management strategies for yellow foxtail should prioritize

preventing seed production over consecutive seasons to reduce its presence in the seedbank. The life cycle of yellow foxtail begins in early spring with seed germination, followed by rapid vegetative growth during late spring and early summer. Flowering occurs in mid-to-late summer, with seeds maturing in early fall. After senescence, the plant dies, leaving behind seeds that replenish the soil seedbank for the next cycle. Since it is an annual, its survival and spread depend entirely on successful seed production and dispersal.

In contrast, knotroot foxtail is a warm-season perennial species that reproduces through both seeds and vegetative rhizomes. While seed production occurs, rhizomes are the primary means of propagation, allowing the plant to overwinter and re-emerge annually (Rector *et al*., 2024). Germination of *S. parviflora* seeds is influenced by multiple environmental factors, including light, soil nitrate levels, and temperature (Mollard and Insausti, 2009; Mollard *et al*, 2007).

In particular, exposure to light and the presence of nitrates have been found to break seed dormancy and promote germination (Martin 1943; Banting *et al*. 1973). These adaptive traits enable knotroot foxtail to survive in both disturbed and moisture-variable environments, roadsides, including ditches, hayfields, and low-lying pastures (Dekker, 2003). Knotroot foxtail, however, follows a perennial life cycle. Seedlings emerge in spring or early summer, and vegetative growth leads to the development of an extensive network of underground rhizomes. In established stands, rhizomes are the dominant mode of spread, often forming dense patches that outcompete desirable forage species (McCullough, 2016). This persistent growth habit makes knotroot foxtail particularly difficult to control with herbicides alone, as treatments targeting aboveground shoots may leave the underground rhizome system unaffected (Rector *et al*., 2024).

Botanical description of yellow and knotroot foxtail

Yellow foxtail is an annual grass characterized by fibrous roots and upright stems that either stand alone or grow in small clusters from the base. The culms often reach heights between 10 and 120 cm and may exhibit bent nodes near the base. Its leaf sheaths are glabrous along the margins, and the ligule is made up of a fringe of joined ciliate hairs, typically about 3 mm tall with roughly 50 hairs per millimeter. Leaf blades are generally 4 to 10 mm wide and can grow up to 30 cm in length, often loosely twisted with a rough upper surface. A distinct feature is the presence of hygroscopic hairs just above the ligule, numbering around 80 per plant and measuring between 4 and 10 mm in length. The inflorescence is a narrow, compact panicle, usually 3 to 10 cm long.

Each panicle branch is very short (under 1 mm), bearing a single fertile spikelet accompanied by a cluster of typically 4 to 12 yellow, orange, or tawny bristles that range from 3 to 10 mm long. The spikelets are planoconvex and 3.0 to 3.5 mm in length. The fruit is an elliptical caryopsis enclosed within the persistent lemma and palea (North Carolina Extension Gardner Plant Tool Box, Gleason and Cronquist, 1963, Hitchcock, 1971; Morrone *et al*., 2014; Crampton, 1974).

Knotroot foxtail is a perennial species with a fibrous root system and distinctive short, swollen rhizomes. The culms emerge singly or in small tufts and typically range from 78 to 120 cm in height with a stem thickness of about 0.5 cm. Leaves measure approximately 14.5 to 18 cm long and around 1 cm wide. Both the leaf blade and sheath are covered with fine hairs, and the ligule is a fringe of hairs without auricles. The upper surface of the leaves is coarse to the touch, while the underside remains smooth. The inflorescence is a densely packed cylindrical panicle, ranging from about 1.3 to 4 cm in length and 0.6 to 2.5 cm in width, with coloration varying from yellowish to purple or brown. Each spikelet is subtended by 4 to 12 bristles, typically between 0.5 and 2.8 cm long (Hitchcock and Chase 1950, Bryson and DeFelice, 2009; Montiel and Mayra, 1975; Rominger, 1959). It is important to note that knotroot foxtail and yellow foxtail share several

overlapping morphological characteristics, which often make them difficult to distinguish. However, this study identified subtle but consistent morphological differences that can be used to separate the two species.

Impact of yellow and knotroot foxtail in forage production systems

Yellow foxtail (*Setaria pumila*) and knotroot foxtail (*Setaria parviflora*) are opportunistic weedy grasses that significantly impact forage systems through both direct competition and indirect effects on forage quality and livestock health (Manthey and Nalewaja 1987; Norris and Schoner 1980; Burrows and Tyrl 2001). Their adaptability to a wide range of environmental conditions and extended germination periods allows them to thrive in hayfields and pastures (Bryson and DeFelice 2009). These species compete vigorously with desirable forage grasses such as bermudagrass and bahiagrass for light, moisture, and nutrients, ultimately reducing forage yield and stand persistence (Lawrence *et al*. 1989). Knotroot foxtail exhibits morphological and genetic similarities to yellow foxtail, often leading to misidentification. This mimicry complicates species-level identification in the field and poses challenges for effective weed management (Rominger, 1962; Chikara and Gupta, 1980). Yellow foxtail, although an annual, can be highly prolific, producing up to 890 seeds per plant (Forcella *et al*. 2000). Larson *et al*. (2016) demonstrated that as few as one foxtail plant m^{-2} reduced forage yield by 25% in the first production year, with losses exceeding 90% at densities above 50 plants m^{-2}.

Similarly, studies in alfalfa systems have shown that yellow foxtail reduces yield and yield quality, dry matter yield and negatively impacts nutritive value (Shahab *et al*., 2025; Renz, 2018). In mid-to-late summer, both species develop bristly seedheads that are unpalatable and potentially injurious to livestock. These bristles, or awns on the seedhead, can cause oral ulcers in horses and

deter livestock from grazing, leading to uneven forage utilization (Kutasi *et al*. 2018; Burrows and Tyrl 2001).

Knotroot foxtail can be infected by the fungus *Beniowskia sphaeroidea*, which also affects other forage grasses. Such infections may reduce forage quality and pose risks to hay production (Taber *et al*., 1978; Brown and Hanlin, 1982). Knotroot foxtail presents greater long-term management challenges due to its rhizomatous perennial growth habit, which allows it to regenerate following mowing or incomplete herbicide control (Griffin and Gunsaulis 2020; Dyer, 2022). Knotroot foxtail frequently grows over a meter tall along roadsides, obstructing drivers' lines of sight and posing safety risks across extensive highway systems in the southeastern U.S. (Israel *et al*. 2014). In Alabama, this species is a troublesome weed in bahiagrass (*Paspalum notatum* Flüeggé) seed production fields, where its similar seed size and maturation timing complicate effective separation during harvest (Wehtje *et al*. 2008).

Control options for yellow and knotroot foxtail

Effective management of yellow foxtail (*Setaria pumila*) and knotroot foxtail (*Setaria parviflora*) in forage systems necessitates an integrated approach that combines non-chemical and chemical strategies. Cultural practices are foundational for long-term suppression of these species. Proper grazing management is essential to maintain forage canopy density, as overgrazed pastures reduce competition and facilitate foxtail establishment (Hoveland *et al*., 1996). Timely mowing before seedhead development can significantly reduce seed production and limit further spread (Lawrence *et al*., 1989). Deep mechanical tillage may also be beneficial, as foxtail emergence declines sharply with increased burial depth seeds buried beyond 5 cm exhibit significantly reduced emergence, suggesting that deep tillage can help deplete the seedbank in heavily infested fields (Dyer *et al*., 2024a, Forcella *et al*., 2000). In addition, maintaining optimal soil fertility

through appropriate nutrient management enhances the competitiveness of desirable forage species, further suppressing weed establishment (Bryson and DeFelice, 2009).

Chemical control of yellow and knotroot foxtail requires careful selection of herbicides based on the lifecycle of the weed and growth stage. Preemergence herbicides such as pendimethalin (Prowl H_2O) at 11.52 kg ha^{-1} (4.2qt/acre) applied in early spring before green-up and rainfall, are effective in suppressing annual foxtail emergence in established forage systems (Russell, 2021). Indaziflam (Rezilon) applied at 0.45 kg ha^{-1} (5 fl oz/acre) or in split doses (3 + 3 fl oz) before weed emergence has shown extended residual control of annual grasses but limited efficacy on established knotroot foxtail that emerge from rhizome (Dyer *et al*., 2024; Russell, 2021). In alfalfa, flumioxazin 0.14 - 0.28 $kgha^{-1}$ (2 - 4 oz/acre) can be used for preemergence suppression of annual foxtail and the length of control can be extended by applying flumioxazin in early spring followed by pendimethalin after first spring harvest (McCullough, 2016). It is important to note that flumioxazin should not be used in mixed stands of alfalfa and forage grasses as it can cause injury to desirable grasses (McCullough, 2016). Additional preemergence herbicide options for managing foxtail in alfalfa forage systems include EPTC (Eptam) and benfluralin (Benefin) (McCullough, 2016)

For postemergence chemical control measures, pastora (nicosulfuron + metsulfuron) at 0.11 kg ha^{-1} (1.5 oz/acre) provides control of seedling annual foxtail in bermudagrass. A sequential program of 1.5 oz Pastora + 8 oz glyphosate followed by 1 oz Pastora 14–16 days later offers up to 70% control of knotroot foxtail but may stunt bermudagrass (Russell and Dillard, 2020). Quinclorac (Facet L) at 5.33 kg ha^{-1} (2 qt/acre) provided 75–80% control of knotroot foxtail for three months after application (Russell and Dillard, 2020). Similarly, quinclorac at 0.84 kg ai/ha plus methylated seed oil at 1% volume/volume has been found to control knotroot foxtail and this

treatment is most effective when applied after the hay cutting (Rector *et al*, 2018). Knotroot foxtail was controlled by 90% at 8 weeks after treatment with a tank mix of imazapic at 0.14 kg/ha and glyphosate at 0.42 kg/ha (Burns, 2006). Hexazinone (Velpar) applied at 5.0 kg/ha (2 qt/acre) in early spring has provided 85–90% foxtail control for up to three months (Russell, 2021). Spot-applied glyphosate (1% solution) in fall is more effective than spring for rhizome kill (McCullough, 2016). Imazamox and imazethapyr is also one of the available postemergence option for suppressing annual foxtails in alfalfa (McCullough, 2016). Diclofop applications at 1.12 kg ha^{-1} proved effective in suppressing knotroot foxtail seed head production in bahiagrass (Wehtje *et al*., 2008).

Mode of action and use of quinclorac and hexazinone in forage systems

Quinclorac is a synthetic auxin herbicide (Group 4) that stands out from other auxins due to its unique ability to control certain grass weeds in addition to broadleaf species. While most auxin-type herbicides primarily affect dicot species, quinclorac is notably effective on grasses (Grossman and Scheltrup 1998). Quinclorac act by mimicking an auxin overdose which affects the phytohormonal system in sensitive plants. This happens through a target-site-based mechanism involving the stimulation of ethylene biosynthesis and the subsequent accumulation of phytotoxic cyanide (Peng and Byer, 2005; Grossmann and Kwiatkowski, 2000).

In susceptible species such as green foxtail, large crabgrass, and broadleaf signalgrass, quinclorac induces the enzyme ACC synthase, which increases ethylene production (Curan *et al*. 2011; Heap and Morrison, 1996). As a by-product of ethylene biosynthesis, cyanide accumulates to toxic levels, leading to cellular damage and inhibited shoot growth. Quinclorac is absorbed through both foliar and root tissues, and it translocate via both xylem and phloem, reaching meristematic tissues where it induces its effects (Grossmann and Kwiatkowski, 2000; Lamoureux

and Rusness, 1995; Koo etal., 1994). It is metabolized only moderately, with the parent compound remaining predominant in both roots and shoots after treatment (Peng and Byer, 2005). In forage production in Southeast United States, quinclorac is labeled for use in established bermudagrass and tall fescue, where it offers selective control of grassy weeds such as foxtails with minimal injury when used according to label directions. Additionally, quinclorac is compatible in tank mixes with several herbicides. Peng and Byer (2005) demonstrated its compatibility in mixtures targeting broader weed spectrums, making it a valuable tool in integrated weed management programs.

Hexazinone is a Group 5 herbicide classified as a photosystem II inhibitor. It works by binding to the D1 protein in the thylakoid membrane, disrupting the electron transport chain during photosynthesis (Teixeira *et al*., 2024). This leads to the formation of reactive oxygen species that damage plant cells and ultimately cause death (Chaudhary *et al*., 2020). Hexazinone is known for its broad-spectrum weed control, including activity on several difficult to manage grass species Ferrell and Mullahey, 2006). In forage systems, it has demonstrated strong potential for managing foxtails. For example, Wehtje *et al*. (2008) reported 75–85% control of knotroot foxtail in bahiagrass pastures following hexazinone application. Similarly, Brighenti, 2024) found that hexazinone suppressed grassy weeds in elephant grass pasture establishment when applied early postemergence. Dyer *et al*. (2024) also reported the ability of hexazinone to reduce foxtail emergence and biomass in bermudagrass systems, especially when timed with rainfall events. Hexazinone possesses both foliar and soil activity (Michael, 2001). After being absorbed through the soil, the hexazinone is transported via the xylem to green tissues, where it disrupts photosynthesis. Crop safety is a critical factor in herbicide selection for forage systems, as selectively managing grassy weeds within grass crops is particularly challenging. Hexazinone is

specifically labeled for use in well-established stands of bermudagrass and bahiagrass, where it can be applied with minimal risk to the forage species. Wehtje *et al*. (2008) observed minimal injury to bahiagrass under favorable condition when hexazinone was used at recommended rates.

Physiochemical properties influencing the performance hexazinone and quinclorac

The behavior and efficacy of herbicides is strongly influenced by their physicochemical properties, which affect their absorption, movement and persistence in the environment (Krähmer *et al*., 2021; Zhang *et al*., 2018). Quinclorac and hexazinone exhibit distinct physiochemical properties that influence their mobility, persistence, and uptake in forage systems. Quinclorac has moderate water solubility (~71 mg/L at pH 7, 20°C) (US EPA, 1998; MacBean, 2008–2010) and a soil adsorption coefficient (K_{oc}) ranging from 41 to 100 mL/g (JMPS, 2002). This suggests that quinclorac binds moderately to soil particles, such that a portion of the herbicide remains free in the soil solution, while the rest is adsorbed to soil surfaces. As a result, its moderate binding allows enough of the herbicide to stay available in the soil solution for plant root uptake, especially under favorable moisture conditions (Chism *et al*., 2017).

Quinclorac also has a pKa of 4.34 (MacBean, 2008–2010; NCBI, 2025), which means that under typical soil pH conditions (generally above 5), quinclorac exists mostly in its anionic (negatively charged) form (JMPS, 2002). This property reduces its attraction to negatively charged soil components like clay and organic matter, further increasing its potential to move through the soil solution (Redeker, 1988). However, sufficient rainfall or irrigation is still needed to move quinclorac into the root zone where it can be effectively taken up by target weeds (Grossmann and Kwiatkowski, 2000; Peng and Byer, 2005). Quinclorac is absorbed through both foliage and roots and is translocated via the xylem and phloem, allowing it to reach meristematic tissues and effectively control actively growing grasses like foxtails and crabgrass (Grossmann and

Kwiatkowski, 2000; Peng and Byer, 2005). Its soil half-life ranges from 20 to 100 days, depending on environmental conditions such as temperature and moisture, providing moderate residual activity with lower concerns for long-term persistence (US EPA, 1998; El-Dars *et al*., 2023; Lym, 2016).

In contrast, hexazinone, a herbicide that belongs to the triazine family in Group 5 with high water solubility, ranging from 29,800 to 33,000 mg/L at 25°C (Bouchard and Lavy, 1985; Senseman, 2007; Shaner, 2014). It has a low soil adsorption coefficient (Koc: ~54–100 mL/g), meaning it binds weakly to soil particles and remains readily available in the soil solution for plant uptake (Shaner, 2014; USDA Forest Service, 2005; Linders *et al*., 1994). It is absorbed primarily through the roots and shoots, and transported via the xylem to photosynthetically active tissues, where it inhibits photosystem II and disrupts photosynthesis (Senseman, 2007; Shaner, 2014). Its soil half-life ranges from 90 to 180 days, particularly under dry or cool conditions, contributing to extended residual weed control (US EPA, 2005; Wehtje *et al*., 2008). However, its high solubility and weak adsorption also increase its risk of leaching, especially in sandy or low-organic matter soils (Tu *et al*., 2001; Bouchard and Lavy, 1985).

Herbicide activation with soil moisture and rainfall

Soil-applied preemergence herbicides require adequate moisture for activation, which allows the herbicide to dissolve into the soil solution and move into the upper 1–2 inches of soil, where it can be absorbed by germinating weed seedlings (Stickler *et al*., 1969; Walker and Roberts, 1975). In dry conditions, herbicide molecules remain adsorbed to soil particles, limiting their mobility and bioavailability, often resulting in delayed or inconsistent weed control (Jordan *et al*., 1968). This challenge is particularly critical in forage systems, where early-season weed suppression is essential to reduce competition and promote healthy forage stand. The amount of

rainfall or irrigation required for effective activation depends on multiple factors, including the herbicide's solubility, adsorption characteristics, soil texture and organic matter content, the sensitivity of weed species, and soil moisture status at the time of application (Hammerton, 1967; Russell *et al*., 1990). Moist soils following adequate rainfall improve herbicide incorporation and enhance the activity of herbicides, whereas dry soils can delay activation, increasing the risk of weed germination before uptake occurs (Caseley, 1987; Anderson *et al*., 1993; Russell *et al*., 1990). When sufficient rainfall is received after application within 7–10 days of application is often sufficient to activate many preemergence herbicides (Johnson and Zimmer, 2023).

In addition to soil-applied herbicides, rainfall also plays a significant role in the performance of foliar-applied herbicides. Rainfall occurring too soon after application before the herbicide is adequately absorbed can wash it off the leaf surface, reducing uptake and leading to poor weed control (Bryson, 1987, 1988; Behrens and Elakkad, 1981). This is why each herbicide has a defined rainfast period, which indicates how long it needs to remain on the leaf surface before becoming washed away by rain. However, under certain conditions, light rainfall or increased post-rain humidity can enhance absorption by softening or hydrating the leaf cuticle, especially in species with waxy or hairy leaves (Bovey and Diaz-Colon, 1969). Prolonged leaf wetness after rainfall can extend the absorption window, promoting uptake. Nevertheless, heavy or early rainfall typically presents a greater risk of herbicide loss (Anderson *et al*., 1993).

Proper herbicide selection based on expected rainfall patterns, soil properties, crop tolerance, and application timing is essential for successful weed management especially in perennial forage systems, where selective grass weed control remains a significant challenge. Understanding how herbicides interact with environmental and edaphic factors enables producers

to make informed decisions regarding product choice, the need for supplemental irrigation, and the timing of application for more consistent and effective weed control

Overview of the Thesis

Knotroot foxtail (*Setaria parviflora* (Poir.) Kerguélen) and yellow foxtail (*Setaria pumila* (Poir.) Roem. and Schult.) are problematic grass weeds in southeastern United States, infesting pastures, hayfields, Turfgrass, roadsides, and unmanaged lands (Bryson and DeFelice, 2009; ACES, 2021; Hitchcock, 1971). These two species are morphologically similar, which often leads to misidentification and confusion in herbicide selection (Darmency and Dekker, 2011). While yellow foxtail is an annual, knotroot foxtail is a warm-season perennial that aggressively invades forage systems and propagates through both seed and rhizomes, making it especially difficult to manage (Bryson and DeFelice, 2009; McCullough, 2016).

Young knotroot foxtail plants may offer acceptable forage value, but rapid seed production, declining quality at maturity, and the potential to injure livestock such as causing mouth ulcers when grazed when knotroot foxtail had formed seedhead underscore the negative impacts of this species on grazing systems (McCullough, 2016; Israel *et al*., 2014). While rhizomes are the primary characteristic distinguishing knotroot foxtail from yellow foxtail (Wang *et al*., 1995), little is known about the timing of its transition from a seedling to a rhizome-producing plant. Understanding this developmental stage is crucial for identifying the optimal period to manage knotroot foxtail seedlings before they establish the perennial structures (rhizome).

Only a few herbicides, such as hexazinone and quinclorac have demonstrated reliable activity against knotroot foxtail in forage systems (Russell, 2022). Both herbicides possess foliar and soil activity, but their success depends heavily on timely rainfall or irrigation to activate them

in the soil and facilitate root uptake. However, the optimal timing of rainfall after application remains unclear, and inadequate moisture during this window could severely reduce herbicide efficacy and allow knotroot foxtail to persist.

Given these biological and management complexities, this thesis addresses two critical research needs. First, it examines the developmental stages of knotroot foxtail from seed to rhizome and explores its morphological differentiation from yellow foxtail. Second, it evaluates the influence of simulated rainfall timing on the performance of hexazinone and quinclorac in controlling knotroot foxtail. Together, these efforts aim to fill essential knowledge gaps and support the development of timely and integrated weed management strategies for effective control of knotroot foxtail in the southeastern United States.

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Chapter II.

Developmental Stages of Knotroot Foxtail [*Setaria parviflora* (Poir.) Kerguélen] from Seed to Rhizome and its Morphological Differentiation with Yellow Foxtail [*Setaria pumila* (Poir.) Roem. and Schult].

Abstract

Knotroot foxtail is a perennial grass weed that reproduces through both seeds and rhizomes. Its close resemblance to yellow foxtail complicates identification and management. Currently, there is no published information on the timing of rhizome development, which could help optimize management strategies. A greenhouse and field study was conducted at Auburn University to investigate the developmental stages of knotroot foxtail and its morphological differences from yellow foxtail. Knotroot foxtail seeds from five populations in Alabama were grown in the greenhouse and later transplanted to the field in a randomized complete block design with five replications. Seedling emergence occurred between 8 and 14 days after sowing. Rhizome formation among 50% of knotroot foxtail plants was observed between 7 and 9 weeks after seed sowing. At the Beef Unit, 50% of the knotroot foxtail population developed rhizomes at plant heights of 12.7–24.1 cm and the 1–2 leaf stage, while at the Turf Unit, 50% rhizome development occurred at 40.6–52.6 cm and the 3–5 leaf stage.

Seedhead formation occurred between 59.8 and 66.8 days after planting. Principal Component Analysis (PCA) using eight morphological traits distinguished the foxtail species. The first two components explained 70.9% of the variation between the two species. PC1 (58.26%) differentiated yellow and knotroot foxtail based on first true leaf width and length, spikelet width

and length, internode length and stem diameter. PC2 (12.64%) was influenced by pedicel length and mature leaf width. Qualitative traits such as leaf margin roughness, leaf color, and seed size also differ between knotroot foxtail and yellow foxtail. This data provides some insight into the biology of knotroot and yellow foxtail. A single morphological trait identified in this study may not be sufficient for distinguishing between yellow and knotroot foxtail, especially at early growth stages. Effective identification may require combining multiple traits to account for subtle differences.

Keywords: Emergence, internode, knotroot, morphometrics, rhizome.

Nomenclature: Knotroot foxtail, *Setaria parviflora* (Poir.) Kerguelen; yellow foxtail, *Setaria pumila* (Poir.) Roem. and Schult.

Introduction

Among species in the Setaria genus, yellow foxtail, knotroot foxtail, green foxtail , and giant foxtail are some of the most troublesome weeds affecting agriculture and managed ecosystems worldwide (Dekker 2003; Holm et al., 1977; Staniforth 1965). These species are highly adaptable and thrive across a broad range of environments, except in regions where extreme cold and persistent ice cover prevent seed germination and plant establishment (Dekker 2003). Knotroot foxtail also known as yellow bristlegrass is originally from the Americas (Barkworth *et al*., 2007; Rominger 1962; Bryson and DeFelice, 2009), while yellow foxtail has its provenance in Eurasia and has spread across Asia, Europe, North America, and the humid regions of Australia. Yellow foxtail is also found in southern Africa, the Caribbean, and the Andean regions of South America (Mohler *et al*., 2021; Dekker 2003).

Among the *Setaria* species present in the southeastern United States, knotroot and yellow foxtail are among the most commonly found and widely distributed, particularly in pastures, hayfields, and highway rights-of-way (McCullough, 2016). These species frequently invade disturbed areas and thrive in managed warm-season grass systems, making them a persistent challenge for land managers and quality forage producers (McCullough, 2016; Bryson and DeFelice, 2009). The ability of these two Setaria species to establish in various environments contributes to their resilience, complicating control efforts and impacting forage quality and availability (Dekker, 2003).

Yellow foxtail is an annual grass, whereas knotroot foxtail is a perennial. Both species reproduce prolifically by seed which facilitate their spread, rapid multiplication, and invasion of new areas (Forcella *et al*., 2000). Their variable emergence timing further enhances their competitive advantage over desirable forage species. Foxtail species produce abundant seeds with

long dormancy, allowing them to persist in the soil for up to 30 years and quickly dominate pastures or hayfields when human activities or environmental disturbances suppress desirable forage species (Dekker, 2003; Dekker 2004; Russell and Dillard, 2020). Additionally, knotroot foxtail also produces rhizomes, which serve as perennating organs that enable asexual reproduction (Doust and Diao 2017). Knotroot foxtail matures more quickly than the other Setaria species and forms seedheads much earlier (June) than the annual foxtails (August to September), giving it a competitive advantage and making it more likely to reduce pasture productivity (Dyer 2025; Rector *et al*., 2018). In many agroecosystems, weedy Setaria spp. are not necessarily the first species to emerge (Buhler *et al*., 1997). However, even in communities where they emerge later, they frequently outcompete other species (Buhler *et al*., 1997).

Knotroot foxtail and yellow foxtail are often unnoticeable in pastures and hayfields when young, as livestock readily graze on them (Israel *et al*., 2014; Marten and Andersen, 1975). However, as the plants mature and develop seedheads, grazing animals such as horses and cattle tend to avoid them. The sharp awns on the seedheads (spikelets) can become embedded in their gums and tongue, causing irritation, infections, and ulcers (Israel *et al*., 2014). When these spikelets are mixed with harvested hay, they can reduce feed intake, lead to weight loss, and negatively impact livestock health (Rector *et al*., 2018). Horse owners avoid buying hay contaminated with foxtail weeds because its high oxalate content makes it unsafe for horses and can lead to kidney failure when consumed (Rahman *et al*., 2012).

One of the key weedy traits exhibited by knotroot foxtail and yellow foxtail is mimicry, the ability of one species to resemble another, which makes identification and management more challenging (Chikara and Gupta, 1980; Rominger, 1962). Knotroot foxtail is often mistaken for yellow foxtail due to their morphological similarities. The presence of rhizomes and other criteria

used to differentiate yellow and knotroot appears late, long after identification is required for weeds management in pasture (Wang, 1994). Therefore, understanding the morphological differences between these two species is crucial for accurate identification and effective management.

More importantly, controlling foxtails at an early growth stage is widely recommended to prevent seed production and reduce future infestations. However, in the case of knotroot foxtail, which has the added ability to form rhizomes, early control becomes even more critical. If left unmanaged, this species not only contributes to the soil seedbank but also establishes a perennial root system that allows it to persist and regenerate even after aboveground removal (Israel *et al*., 2014). Rhizome formation means that delayed control efforts may be ineffective, as the weed can continue spreading underground even if seed production is suppressed.

Effective weed management strategies rely on a thorough understanding of a species' life cycle. Recent studies have identified distinct morphological features that provide insights into the biology of yellow and knotroot foxtail (Dyer, 2025; Joseph *et al*., 2024). While research on the biology and management of annual *Setaria* species in row crops and pastures exists, there is limited information on the early development of knotroot foxtail, particularly its transition from seedling to rhizome production. This knowledge gap limits the ability to implement effective control measures during its most vulnerable growth stages. Additionally, some of these studies have focused on Setaria species in Turfgrasses rather than pastures (Joseph *et al*., 2023), even though foxtails grow differently in forage ecosystems due to varying levels of plant competition. In pastures or hayfields, knotroot and yellow foxtail compete with neighboring grasses, which influences their growth patterns. While physical traits are the main method for identifying foxtail species in the field, their reliability can vary because the same species may grow differently in

different environments (phenotypic plasticity). This natural variation, common in weeds, makes identification more challenging. Therefore, it is imperative to accurately identify the morphological differences between the two species at an early stage to better understand their biology. To address this gap, this study aims to determine how long it takes for knotroot foxtail to transition from seedling to rhizome formation. Since knotroot foxtail is a perennial species, we hypothesize that it will develop rhizomes within the first year after emergence. Specifically, this research will, (1)Determine the time required for knotroot foxtail to transition from a seedling to a mature plant capable of producing rhizomes and identify key morphological changes during the vegetative and reproductive stages of knotroot foxtail.(2)Compare and document the morphological differences between yellow foxtail and knotroot foxtail in forage systems.

Materials and Methods

Seed Collection and Processing

Mature seedheads of knotroot foxtail were collected in late summer 2023 from multiple locations across Alabama state, including Bullock, Cullman, Geneva, Macon, and Tallapoosa counties (Figure 1), representing diverse populations within the state. The collected seedheads were air-dried, and the seeds were removed from the spikelet. The threshed seeds were stored in a refrigerator at 8°C until planting in 2024.

Seed Germination and Greenhouse Establishment

Before establishing the main experiment in 2024, a preliminary germination trial was conducted in a controlled greenhouse environment at Auburn University (32.58807°N, 85.48890°W) to determine the most effective pre-germination treatment for knotroot foxtail seeds. This trial was necessary as initial seed planting did not result in emergence. A total of 60 seeds were mechanically scarified using a coarse sanding sponge to remove awns and were divided into three treatment groups, with 20 seeds per treatment: mechanical scarification alone, scarification followed by a 30-minute warm water soak (40–49°C) inside organza bags, and scarification followed by a 30-minute soak in 1% bleach inside organza bags. Based on observed germination success, the scarification followed by a 30-minute warm water soak treatment was identified as the most effective, resulting in 98% seed emergence, and was used for all subsequent plantings in the study.

Using this effective germination method, a total of 1,440 seeds, with 290 seeds per population, were sown at a depth of 1 cm in 72-cell greenhouse plug trays on May 23, 2024. The

trays were filled with Miracle-Gro® Potting Mix, which contains 0.21% total nitrogen (N), 0.11% available phosphate (P_2O_5), and 0.16% soluble potash (K_2O). Each cell within the 72-cell trays was planted with four seeds. No nutritional deficiency symptoms were observed on the plants throughout the experimental duration in the greenhouse, therefore, fertilizer was not applied. Seedlings were grown in the greenhouse for five weeks before being transplanted into field plots. The greenhouse was maintained at day/night temperatures of 22.2–25°C with supplemental lighting to achieve a 14-hour photoperiod. Weather parameters, including temperature, and relative humidity (Figure 2), were recorded throughout the growing period. Daily maximum and minimum air and soil temperatures (Figure 2) were obtained from the Vantage Pro2 Weather Station, which was located near the greenhouse.

Field Establishment and Experimental Design at the Two Location Sites in 2024

The field was first rototilled and rolled with a water ballast roller to firm the soil prior to planting. Knotroot foxtail seedlings, grown in the greenhouse from seed, were transplanted into field plots five weeks after sowing at two locations at Auburn University (Turf Unit – 32.57695°N, 85.50130°W; Beef Unit – 32.58544°N, 85.50556°W). Soil characteristics at both locations are presented in Table 2-1. One day following transplanting, a premix preemergence herbicide containing dimethenamid-P and pendimethalin (Freehand 1.75G) was applied at a rate of 10.43 kg ai ha^{-1}. After herbicide application, the plots were irrigated to activate the herbicide and enhance its effectiveness in controlling emerging weeds.

The experimental layout was a Randomized Complete Block Design (RCBD) with five populations as treatments. Each block contained six plants per population, resulting in a total of 30 plants per block. The experiment was replicated five times, with blocks serving as replicates. The field site was maintained under irrigation as needed to ensure optimal growth conditions. One

week after transplanting, belowground measurements were obtained by carefully uprooting two plants from each of the five populations at each location. Plants were excavated weekly for 12 consecutive weeks using a hand trowel to minimize root damage. Excess soil was gently shaken off, and the rhizome-root stubble was washed under a stream of water to remove remaining soil particles before being air-dried. The aerial portions of the plants were clipped approximately ½ inch above the ground using scissors. The excavation procedure followed the method described by Taylor (1986) for studying root systems. After air-drying, rhizome-root fresh biomass and root length were measured.

Morphology Study in 2024

In this study, morphometric characteristics of knotroot foxtail and yellow foxtail were evaluated at different growth stages to compare species traits. Seeds of yellow foxtail were collected from multiple populations across Macon County, Lee County, and Winston County in Alabama, along with an accession from the USDA U.S. National Plant Germplasm System. Knotroot foxtail seeds were obtained from the same populations used in the seed-to-rhizome development study. A total of 80 seeds (40 seeds from each species) were sown on June 11, 2024, at a depth of 1 cm in two 4-cell greenhouse plug trays. The trays were filled with Miracle-Gro® Potting Mix, which contains 0.21% total nitrogen (N), 0.11% available phosphate (P_2O_5), and 0.16% soluble potash (K_2O). Approximately 10 seeds were planted per cell. The greenhouse was maintained at day/night temperatures of 22.2–25°C with supplemental lighting to provide a 14-hour photoperiod. A total of 60 plants (30 knotroot foxtail and 30 yellow foxtail) were measured.

Seedlings grown in the greenhouse were used to assess traits identifiable at early growth stages, while mature plant traits were measured *in situ* at various developmental stages. Mature plants were sampled from five field locations in Lee County, Alabama: 32.58246°N, 85.50297°W;

32.58544°N, 85.50556°W; 32.58547°N, 85.50561°W; 32.43754°N, 85.93039°W; and 32.57697°N, 85.50138°W. Both quantitative and qualitative traits were recorded based on standardized grass morphology descriptors (Hitchcock and Chase, 1950), providing a comprehensive assessment of species differentiation across developmental stages.

Data Collection

For the seed-to-rhizome study, days to emergence were recorded for each knotroot foxtail population and documented as the number of days from planting to emergence. Seedling emergence was defined as the coleoptile being visible at the soil surface and was also recorded daily from May 23 through June 7, 2024. The number of newly emerged seedlings in each plug cell was counted and recorded at each observation time. Emergence was assessed as the proportion of seedlings emerged relative to the total number of seeds planted. After counting, emerged seedlings were not removed, allowing for continued tracking of their development. Data collection continued until no new emergence was recorded over a consecutive 15-day period, which was used as the termination criterion for emergence monitoring, indicating maximum emergence had been reached. Emergence counts were converted to cumulative emergence, where the total number of emerged seedlings on each day was summed over time.

Following emergence, other plant vegetative traits such as plant height, leaf stage, rhizome formation (0 = absence, 1 = presence), fresh rhizome-root biomass (rhizome with roots still attached and not separated) and root length were collected. Days to seedhead formation was recorded as the first visible sign of the inflorescence while still enclosed within the sheath of the flag leaf. Daily observations began 6 weeks after planting, and data were recorded for individual plants within each population until all plants had initiated flowering.

For the morphological differentiation study, various quantitative and qualitative traits were measured at different growth stages of the two foxtail species. These traits were documented and described based on visual observations, following standard taxonomic descriptors. The identification keys for the morphometric traits were obtained from the Manual of the Grasses of the United States (Hitchcock and Chase, 1950).

Statistical Analysis

The number of days to seedling emergence for the different knotroot foxtail populations was modeled using a three-parameter Weibull Type I function, with the lower limit fixed at zero. The model was fitted using the *drm*() function from the *drc* package in RStudio and is represented by Equation 1 (Ritz *et al*., 2015; Wu *et al*, 2007; Weibull 1959):

$$f(x) = 0 + (d - 0)\exp\{-\exp[b(\log(x) - e)]\} \quad [1]$$

Where $f(x)$ is the cumulative proportion of seedlings emerged at time t (days), e represents the time (days) at which 50% emergence is reached, b is the shape or slope parameter (describes how steep the curve is), and d is the maximum emergence recorded. Similarly, the time to rhizome formation among the five knotroot foxtail populations was analyzed using a three-parameter Weibull Type I model, with the upper limit fixed at 1 and the lower limit fixed at 0, following the method described by Ritz et al. (2015). The model is expressed as:

$$f(x) = 0 + (1 - 0)\exp\{-\exp[b(\log(x) - e)]\} \quad [2]$$

Where $f(x)$ is the probability (or proportion) of plants not yet forming rhizomes at time t, d is the upper asymptote, e represents the time to 50% rhizome formation, b is the slope or shape parameter that determines how quickly rhizomes are formed relative to time where c is the lower limit. A

nonlinear three-parameter log-logistic regression model was used to evaluate the relationship between rhizome formation and plant traits, including plant height, leaf stage, fresh rhizome-root biomass, and root length. The model was fitted using the *drm()* function from the *drc* package in RStudio. To account for potential differences between locations, separate curves were estimated using the *curveid* argument, which allowed the parameters to vary by location (Oddi et al., 2019; Ritz et al., 2015; Aggrey, 2009; Lindstrom and Bates, 1990).

$$y_{ij} = \frac{1}{1+\exp\left[b_i\left(logx_j - loge_i\right)\right]} \quad [3]$$

where y_{ij} is the probability of rhizome formation for observation j at location i, x_j represents the plant trait of interest, including plant height, leaf stage, fresh rhizome-root biomass, or root length, b_i is the slope of each curve, d is the upper limit (fixed at 1), and e_i is the inflection point for location i (which represents the value of "x" at which the probability of rhizome formation reaches 50%.

Likelihood Ratio Tests (LRT) were used to determine whether the inclusion of population, location, or their interaction significantly improved the model fit compared to a reduced model without these terms. After selecting the model structure, model fit was evaluated using root mean square error (RMSE) and the coefficient of determination (R^2) to ensure the models adequately described the data (Knezevic *et al*. 2002; Ritz *et al*. 2015). Additionally, the similarity between parameter estimates of both the three-parameter logistic and Weibull models fitted for each plant trait was assessed using pairwise comparisons with the *compParm*() function from the *drc* package. Differences between parameter estimates were considered statistically significant when the P-value was ≤ 0.05 (Onofri *et al*. 2022; Ritz *et al*. 2015). Data on days to seedhead formation among

knotroot foxtail populations were analyzed using a linear mixed-effects model, where population was treated as a fixed effect and location as a random effect. Mean comparisons among populations were conducted using Tukey's Honest Significant Difference (HSD) test at a 5% significance level.

Principal Component Analysis (PCA) was performed to identify key morphometric traits contributing to the variation between yellow foxtail and knotroot foxtail, with the aim of determining phenotypic variability and identifying morphological traits responsible for these differences (Kenkel *et al*., 2002). The selection of principal components (PCs) for the PCA was based on the Kaiser criterion, which retains components with eigenvalues greater than 1, as shown in the kaiser criterion plot and the scree plot (Figure 2-10) (Field and Miles, 2005; Hill and Lewicki, 2005). The biplot (Figure 2-11) was used to visualize how the different traits contributed to the separation between yellow foxtail and knotroot foxtail. The first two principal components, which explained the majority of the variation, were plotted to illustrate the phenotypic divergence between the two species (Johnson, 1998; Manly, 1994). All statistical analysis was performed using the R Studio (version 2023.3.0.386).

Results

Days to seedling emergence after planting

Emergence data were recorded for seeds grown in the greenhouse in 2024. Variations in emergence patterns were observed among the knotroot foxtail populations that were studied. The estimated time for 50% seed emergence (E_{50}) and 95% seed emergence (E_{95}) was different for each population (Table 2-2). The estimated time for 50% emergence for population A is 8.96 days while it took 13 days for 95% of the planted seeds to emerge. Fifty percent of the seeds planted for

Population B emerged after 9.32 days after sowing, while it took 12.99 days for 95% of the seeds to emerge. Population C had a similar emergence pattern, with 50% emergence at 9.41 days and 95% emergence at 13.08 days. Population D recorded 50% and 95% seed emergence at 9.31 and 12.52 days respectively, while the estimated days to 50% and 95% seed emergence in population E is 9.39 days and 13.67 days. Overall, the time required for 50% to 95% seed emergence across all knotroot foxtail populations that were used in this experiment ranged from 9 to 14 days highlighting variability in their germination timing (Figure 2-4 and Table 2-2).

Variation in emergence timing has been reported by Manthey and Nalewaja (1987); Norris and Schoner (1980) and Stoller and Wax, (1973), who reported the adaptability and extended germination period of knotroot foxtail under varying environmental conditions. Investigations on seedling emergence for Setaria species by Amini *et al*., 2015 revealed that Setaria species show considerable variation in both the timing of spring emergence and the frequency of emergence events throughout the growing season, which can enhance the persistence of different Setaria species. Dekker, 2003 also reported that Setaria species have a flexible and variable seedling emergence pattern, which gives them a competitive advantage to avoid early-season competition, adverse weather conditions, or control measures such as tillage, mowing and herbicide applications. Several studies have reported that weedy foxtail seeds exhibit a high degree of variability in dormancy and emergence rates, enabling them to germinate at specific times across extended periods ranging from a few hours to several decades (Atchison, 2001; Dekker *et al*., 1999; Forcella *et al*., 1992, 1997; Martin, 1943).

Time to rhizome formation

A likelihood ratio test was used to compare three-parameter Weibull type II regression models to determine whether the timing of rhizome formation differed between populations and

locations. The test revealed a significant improvement in model fit when the interaction between location and population was included ($p = 0.011$), indicating that the effect of population on rhizome formation timing varied by location. Therefore, time to rhizome formation data were reported separately for each location.

At the Turf unit, 50% of knotroot foxtail in Population A developed rhizomes, by 7.69 weeks, 95% of the plants developed rhizomes by 7.84 weeks (Table 2-3). Rhizome formation was observed in 50% of knotroot foxtail in population B in 8.02 weeks, while most (95%) of the population has developed rhizome in 11. 67 weeks. For plants in population C, 50% of the plants developed rhizome at 6.96 weeks, and 95% formed rhizome earlier than other populations as they formed rhizome in 7.29 weeks. It took 8.77 weeks for 50% of knotroot foxtail plants in population D to form rhizome while 95% of the knotroot foxtail formed rhizome at 13.31 weeks (Table 2-3). Fifty percent of plants in Population E developed rhizome in 8.97 weeks and 95% of the population formed rhizome in 9.31 weeks. At the Beef location, 50% of knotroot foxtail plants in population A formed rhizomes by 8.10 weeks after planting, while rhizome formation has occurred among 50% of populations B, C, D, and E formed rhizomes at 7.93, 6.56, 9.67, and 6.68 weeks, respectively. By 10.81 weeks, 95% of plants in population A had developed rhizomes, while rhizome formation was observed in 95% of plants in populations B, C, D, and E had developed rhizomes at 13.11, 6.65, 9.89, and 6.84 weeks, respectively (Table 2-3). Based on the results, it was observed that despite differences in locations, 50% of the population has developed rhizome within the range of 6 to 8 weeks after seed sowing. while 95% of the knotroot foxtail population has transitioned into rhizome formation around 9 to13 weeks after planting (Table 2-3). Despite these differences in timing of rhizome formation, all plants across populations had formed rhizomes by the end of the experiment at 16 weeks after seed sowing.

These results indicate that the timing of rhizome initiation varies among knotroot foxtail plants and can occur at an early growth stage of the plant after seedling emergence. A recent study by Dyer *et al*. (2024) also reported that rhizome formation in knotroot foxtail began between the fourth and fifth month after planting (approximately 16 to 20 weeks). Similar rhizome formation patterns have been observed in other perennial grasses, such as johnsongrass, where rhizomes formation occurred as early as 18 days after emergence in half of the plant population examined within the first year (McWhorter, 1961). In cogongrass (Scientific name?_), Holm *et al*. (1977) observed rhizome development 4 weeks after germination, while Shilling *et al*. (1997) reported rhizome formation at 8 weeks. Tominaga (2003) further showed that cogongrass seedlings can produce a large number of rhizomes within the first year of establishment.

This variability in rhizome formation is a common trait in weedy perennial grasses such as knotroot foxtail, allowing them to spread and persist in diverse environments (Mohler *et al*., 2021; Darlington, 1847; Huerd and Moncada, 2001). The ability of individual plants to initiate rhizomes at different times gives the perennial grass species a competitive advantage and makes their management more challenging in both agricultural and natural ecosystems (Kraehmer *et al*., 2012).

Relationship between rhizome formation, plant height, and leaf stage of knotroot foxtail

Relationship between rhizome formation, plant height, and leaf stage of knotroot foxtail

The proportion of knotroot foxtail plants that formed rhizome increases with increasing plant height and leaf stage. A significant interaction between population and location was observed in the relationship between rhizome formation and plant height ($P < 0.001$), indicating that the plant height at which rhizome formation occurred varied across locations and among populations within each location. At the Beef unit, the plant height at which 50% of knotroot foxtail plants had

formed rhizomes (RF_{50}) ranged from 12.7 to 24.1 cm, while the height required for 95% rhizome formation (RF_{95}) ranged from 52.7 to 66.2 cm (Table 4-4). In contrast, at the Turf unit, RF_{50} values were higher, ranging from 40.6 to 52.6 cm, and RF_{95} values ranged from 64.6 to 111.6 cm (Table 4-4).

A significant interaction between population and location was also observed in the relationship between rhizome formation and leaf stage ($P < 0.001$). At the Beef unit, the leaf stage at which 50% of knotroot foxtail plants had formed rhizomes (RF_{50}) ranged from 1 to 2 leaf stage, while 95% rhizome formation (RF_{95}) occurred between 3 and 6 leaf stages (Table 4-5). In contrast, at the Turf unit, RF_{50} values were higher, ranging from 3 to 5, and RF_{95} values extended from 7 to 11 leaf stages (Table 4-5). As the aboveground biomass of knotroot foxtail at both locations increases, the proportion of knotroot foxtail that formed rhizome also increases, and this might be as a result of enhanced translocation of assimilates from the shoots to the roots, supporting rhizome initiation and development.

This process likely occurs as the plant matures and photosynthesizes, directing more resources toward rhizome development. Previous research with johnsongrass [*Sorghum halepense* (L.) Pers.] revealed that increase in aboveground biomass resulted in increased rhizome initiation and growth (McWhorter, 1961; Horowitz, 1972). Keisling (1980) also observed that rhizome initiation in coastal bermudagrass is highly dependent on the aerial portions of the plant for the supply of metabolites. When aboveground biomass is poorly developed, the limited translocation of these essential compounds likely restricts rhizome growth and development.

These data revealed that greater plant height was observed at the Turf unit compared to the Beef unit, despite plants originating from the same populations. This variation may be attributed to differences in soil properties between the two sites (as shown in Table 2-1), which likely

influenced the development of the knotroot foxtail plants grown in these sites. These environmental differences may have triggered phenotypic plasticity, allowing knotroot foxtail plants from the same population to grow differently depending on their environment. The Turf unit consists of loose, sandy soil with better drainage and aeration, which likely promoted deeper root penetration and more efficient nutrient uptake supporting greater plant height and leaf expansion. In contrast, the soil at the Beef unit experimental station site where the knotroot foxtail plants were grown has high soils in organic matter but with more presence of coarse fragments and compaction which may influence root expansion and limited water infiltration in some areas. This is in consistent with Hume, 1982 who reported that nutrient availability, soil fertility, and soil pH play a critical role in the size and growth of Setaria species. Stoller and Sweet (1987) reported that different environments can significantly affect the physiological responses of a plant ecotype. This is further supported by Pigliucci (2001), Alex et al. 1972; Orwick and Schreiber 1975: Nadeau and Morrison 1983, who noted that the size and reproductive capacity of Setaria plants are highly plastic, adjusting in response to environmental factors such as light, water, gases, nutrients, temperature, and neighboring plants.

The results further suggest that knotroot foxtail can form rhizomes regardless of environmental conditions that might affect plant height and leaf stage and this is likely due to its ability to adapt and survive in different environments as it continues to develop key growth stages even under suboptimal growing conditions. Dekker (2023) reported that knotroot foxtail can form rhizome in relatively shallow soil depths (<2.5 cm). This trait allows knotroot foxtail to persist and spread in the field. Understanding these growth patterns and environmental responses can help refine management strategies in pastures and hayfields by ensuring that control measures are

implemented early before seedlings reach the developmental stage at which rhizome formation typically begins.

Relationship between rhizome formation, root length and fresh rhizome-root biomass of knotroot foxtail

A significant population × location interaction was observed in the relationship between rhizome formation and belowground traits, including root length ($P = 0.0018$) and fresh rhizome-root biomass ($P < 0.001$), indicating that the root length and biomass at which for rhizome is developed varied between locations and among populations. At the Beef unit, the root length at which 50% of plants had formed rhizomes (RF_{50}) ranged from 3.9 to 7.6 cm, while RF_{95} values ranged between 13.2 to 20.5 cm (Table 4-6). At the Turf unit, RF_{50} values were generally greater, ranging from 7.7 to 12.3 cm, with RF_{95} values within the range of 21.9 cm reaching as high as 38.7 cm, suggesting that longer roots were associated with rhizome development under turf conditions (Table 4-6). Similarly, the relationship between rhizome formation and fresh rhizome-root biomass differed across locations. At the Beef unit, RF_{50} values ranged from 11.0 to 31.5 g, while RF_{95} values ranged from 20.8 to 49.0 g (Table 4-7). Also at the Turf unit, rhizome formation occurred at higher biomass compared to the Beef unit, with RF_{50} values between 17.5 and 44.3 g, and RF_{95} values ranging from 21.7 to 61.1 g (Table 4-7).

This suggests that soil conditions at each location (Turf and Beef unit) influence root growth and rhizome formation. Knotroot foxtail plants grown in the Turf unit are likely to experience more favorable conditions for growth, which support enhanced belowground development and greater root formation compared to those grown in the Beef unit. A study by Mohler et al. (2021) and Buhler and Mester (1991) reported that foxtail species can grow across a wide range of soil textures, but their growth patterns vary depending on soil type. Nadeau and

Morrison (1986) found that the root length of green foxtail was approximately 1.5 times longer in soils with favorable properties compared to those under reduced moisture conditions. Knotroot foxtail plants at the Turf unit has greater vigorous root growth as their above ground biomass is more than those at the Beef unit and this has significant effect on their root growth. This supports earlier findings that vigorous shoot growth is positively correlated with root development in four different Setaria species (Evetts and Burnside, 1973; Orwick and Schreiber, 1975).

Despite the differences in root length and rhizome-root biomass between the two locations, root length and biomass consistently increased across all knotroot foxtail populations as the proportion of plants forming rhizomes increased over time. Dyer et al. (2024) reported that as knotroot foxtail begins forming rhizomes, it is accompanied by a steady increase in belowground biomass. Over time, this biomass surpassed that of other foxtail species such as green, yellow, and giant foxtail, suggesting a shift from fine roots to storage structures like rhizomes. This sustained investment in belowground growth is likely to contribute to the competitive advantage of knotroot foxtail in mixed plant communities.

Days to Seedhead Formation

The time to seedhead formation in knotroot foxtail did not differ between locations (P = 0.99), however, significant differences were observed among populations as shown in Table 2-5 ($p < 0.001$). As a result, time to seedhead formation was reported among the population (Table 2-6). The number of days required for the transition from vegetative to reproductive growth varied, with Population C taking the longest time to flower (67 days), followed by Population E (65 days), Population B (63 days), and Population D (60 days). It took 60 days for knotroot foxtail plants in population A to form seedhead (Table 2-6). Based on the results from this study, seedhead formation in the knotroot foxtail population occurred between 60 and 67 days after planting. These

findings align with Dyer *et al*. (2024), who reported that the first flowers in knotroot foxtail appeared approximately 60 days after planting.

The differences in days to seedhead formation among knotroot foxtail populations may affect seed production and dispersal. Populations that produce seedheads later have a longer dispersal period, increasing the number of seeds added to the soil seedbank (Forcella *et al*., 1997). This extended seed rain can improve seed survival, cause variation in emergence, and make weed control more difficult, ensuring knotroot foxtail persist and spread (Forcella *et al*., 2000). It is important to note that the variation in time to seedhead formation is not dependent on the location of the seed source. Instead, it could be attributed to environmental factors or ecotypic differences among populations, such as responses to photoperiod, temperature, soil conditions, nutrient availability, light quality, and plant competition. As a result, future studies could investigate how these environmental cues influence seedhead development in knotroot foxtail.

Morphological differentiation between yellow foxtail and knotroot foxtail

Quantitative Traits used for distinguishing yellow and knotroot foxtail

Principal Component Analysis (PCA) was conducted to identify key morphometric traits that differentiate knotroot foxtail from yellow foxtail. A total of nine principal components (PCs) were generated, each accounting for a portion of the total variability in the dataset. However, based on the Kaiser criterion plot (Figure 2-9) and further validated by the scree plot (Figure 2-10), only the first two principal components (PC1 and PC2) were selected for further interpretation, as they had eigenvalues greater than 1 (Silva *et al*, 2020; Braeken and Van Assen, 2017; Kassambara, 2017; Glorfeld 1995; Kaiser, 1960). The eigen values for PC1 and PC2 were 5.24 and 1.14, respectively, confirming their significance in explaining species-level variation. Together, these

two principal components accounted for 70.86% of the total variation in the dataset collected on the morphometric traits used to distinguish yellow foxtail from knotroot foxtail.

The PCA biplot (Figure 2-11) provided a visual representation of how knotroot and yellow foxtail samples are separated based on PC1 and PC2. The PCA scores obtained from the PCA Analysis, as illustrated in the biplot, separates knotroot foxtail and yellow foxtail along the PC1 axis. Plant samples with higher PC1 scores are knotroot foxtail and are clustered on the right side of the plot, while yellow foxtail, with lower PC1 scores, is positioned on the left of the PCA biplot (Figure 2-11). Additionally, the PCA loadings indicate how strongly each trait contributes to PC1 and PC2 (Table 2-7). Traits with higher absolute loadings exert a greater influence on the principal components. PC1, which explained 58.26% of the total variation, was primarily influenced by spikelet width (0.43) and first true leaf length (0.35), both with positive loadings. This suggests that knotroot foxtail, which had higher PC1 scores, tends to have wider spikelets and longer first true leaves. In contrast, traits with negative loadings on PC1 including spikelet length (-0.35), internode length (-0.43), stem diameter (-0.43), and first true leaf width (-0.43) were more associated with yellow foxtail, indicating that plants on the lower end of PC1 tend to have longer spikelets, longer internodes, thicker stems, and broader first true leaves. PC2 accounted for an additional 12.64% of the total variation and was most influenced by length of pedicel (0.712) and width of mature leaf (-0.646). Unlike PC1, PC2 captured within-species variation rather than major species-level differences, indicating that these traits may vary among individuals but do not strongly differentiate knotroot from yellow foxtail.

The traits identified by Principal Component 1 (PC1) played a major role in the morphological classification and differentiation between knotroot foxtail and yellow foxtail. Descriptive statistics for these traits are presented in Table 2-9. The first true leaf length was greater

in knotroot foxtail (4.54 - 5.57 mm) than in yellow foxtail (3.53 - 4.95 mm), while first true leaf width was wider in yellow foxtail (3.00 - 3.69 mm) compared to knotroot foxtail (0.52 - 1.15 mm) (Figure 12). Internode length was significantly longer in yellow foxtail (9.73 - 17.92 cm) than in knotroot foxtail (2.59 - 5.24 cm). Similarly, the stem diameter was wider in yellow foxtail (4.57 - 4.85 cm) than in knotroot foxtail (1.85 - 2.4 cm). Spikelet width was greater in knotroot foxtail (22.05 - 26.95 mm) compared to yellow foxtail (9.72 - 10.76 mm), while spikelet length was longer in yellow foxtail (67.78 - 107.73 mm) than in knotroot foxtail (54.7 - 71.88 mm) (Table 2-8).

These morphological differences highlight key structural traits that distinguish between the two species and may influence their growth, competitiveness, and reproductive strategies in the field. These results correspond to observations of Joseph *et al*. (2023), Hitchcock and Chase (1950) and Hubbard (1954) who found spikelet length as differentiating traits for yellow and knotroot foxtail. Additionally, greater internode length was reported for different yellow foxtail biotypes by Schoner *et al*. (1978). The greater internode length observed in yellow foxtail may contribute to its taller appearance compared to knotroot foxtail. This aligns with the findings by Dyer *et al*. (2024) who previously reported that the height of yellow foxtail was greater than knotroot foxtail three months after emergence. Barkworth *et al*., (2007) found that the width of leaf blades of knotroot foxtail have a scabrous, adaxial surface that ranges from 6.0 to 25.0 cm long with a width of 1.9 mm, whereas yellow foxtail has a slightly wider leaf blade (4 to 10 mm). This disparity in leaf width and length might be due to the effect of varying environmental conditions on the growth and development of the plants. Thus, leaf width or length might not be a reliable distinguishing characteristic for identification purposes (Bryson and DeFelice 2009).

Distinguishing qualitative traits between yellow and knotroot foxtail

In addition to the presence or absence of rhizomes, other distinguishing qualitative traits that were evaluated to distinguish knotroot from yellow foxtail includes leaf margin roughness. The leaf blade margins of yellow foxtail are rougher along the edges compared to knotroot foxtail. Another difference observed was that the seeds of yellow foxtail appeared larger than those of knotroot foxtail across all populations (Figure 2-12). Leaf color also varies between the two species: the leaves of yellow foxtail are predominantly green, whereas knotroot foxtail generally has bluish-green leaf coloration.

Similar observations were made by Mohler *et al*. (2021), who reported that bristly foxtail (*Setaria verticillata*), a weak-stemmed and lodging-prone species, also possesses blue-green foliage, further differentiating it from yellow foxtail. Although growth habit (decumbent vs. upright) was considered, it did not consistently differentiate the two species, particularly in forage grass ecosystems, where these species compete with other grasses, both yellow foxtail and knotroot foxtail can grow either upright or decumbent (Figure 15). This observation partially contrasts with Bryson and DeFelice (2009), who described knotroot foxtail as having the ability to grow either upright or decumbent, reaching heights of up to 1.2 m, while yellow foxtail was predominantly upright. Following these varied observations in qualitative traits, it is important to note that no single trait is sufficient for accurate identification of these two species. Instead, a combination of multiple morphological characteristics must be considered, as previously emphasized by Hubbard (1954).

Similar Qualitative traits between yellow and knotroot foxtail

Both knotroot foxtail and yellow foxtail share several morphological traits, making species identification challenging. The following qualitative traits were recorded

Vegetative characteristics

Leaf sheath shape (keeled and hairless for both species), leaf sheath type (flattened and sharply creased in both species), Sheath margin is split with overlapping margins, leaf edges (not hairy for both species), midrib (less prominent in both species), leaf hair (sparse long silky hairs on the upper surface of the leaf and close to the base of the leaf blade around the ligule region), leaf angle are ascending, , leaf shape (flat for both species), leaf arrangement (alternate for both species), leaf collar type and color (green and continuous), stem color (both species may or may not have red-tinged color near the base of the stem), stem texture (the texture of the stem is smooth for both species), the ligule are hairy and rounded for both species, leaf surface has a dull glossiness for the two species. Both species have clumping growth habit.

The similar morphological features observed in yellow foxtail and knotroot foxtail in this study align with previous reports by Dwayne and Kerner (2003), Mohler *et al*. (2021), Dekker (2001), Dyer *et al*. (2024), and Hitchcock and Chase (1950), which extensively describe the overlapping traits that make species differentiation difficult. These studies highlight similarities in leaf sheath shape, leaf surface texture, inflorescence structure, and overall growth habit, emphasizing that individual traits alone are insufficient for clear identification. Instead, species recognition requires an integrated assessment of multiple characteristics.

Reproductive characteristics

The inflorescence of both yellow and knotroot foxtail are erect, cylindrical and positioned at the top of stem, the inflorescence forming spike-like panicles. The panicle of both species has purple or yellow bristles, and at maturity, the seeds within the spikelets develop bristly awns. The caryopsis (seeds) is enclosed within the entire spikelet and as the plant reaches maturity, the awns

transition to a yellow color (Figure 2-16). Similar descriptions of yellow foxtail and knotroot foxtail inflorescences have been reported by Dwayne and Kerner (2003), Bryson and DeFelice (2009), Barkworth *et al*. (2007), and Hitchcock and Chase (1950)

Below ground characteristics

Both species exhibit a fibrous root system; however, knotroot foxtail also develops rhizomes, distinguishing it from yellow foxtail. This key difference has been well-documented by Hitchcock (1935), Rominger (1962), and Hitchcock and Chase (1950), with the presence of rhizomes classifying knotroot foxtail as a perennial species, in contrast to yellow foxtail, which is considered an annual due to the absence of rhizomes. Dyer *et al*. (2024) also reported differences in root length and biomass between the two species, although these traits are significantly influenced by soil conditions and other environmental factors.

Practical implications and future research

This study provides insights into the biology, development, and species differentiation of knotroot foxtail, a perennial grass weed in forage systems. Variation in seedling emergence was observed among knotroot foxtail populations, 50% of the knotroot foxtail population emerged by 8 days after sowing, while 95% of the population emerged by 14 days under an average temperature of 22.2–25°C. This non-uniform emergence pattern may allow late-emerging cohorts to escape early-season control measures, thereby continuing to compete with desirable forage species throughout the growing season.

As such, understanding the emergence timing of knotroot foxtail is critical for designing season-long management programs. Timely and repeated control strategies are necessary, and integrated weed management plans should include extended monitoring and follow-up treatments

early and later in the growing season to effectively target late-emerging plants. To build on this understanding, future research should investigate which of the knotroot foxtail propagules (seeds or rhizomes) emerge early in the season, as this could further improve the timing of control efforts. Additionally, to better manage mixed infestations of knotroot and yellow foxtail, future studies could also examine which foxtail species (knotroot or yellow foxtail) emerge earlier in the season and identify the environmental factors that influence their emergence timing.

This study also found that rhizome formation can begin as early as 7 weeks after emergence, with 95% of plants studied forming rhizomes by 13 weeks. Also, the proportion of plants forming rhizomes increased with plant height and leaf stage and rhizome formation was observed in plants as short as 12 cm with just two leaves. This provides a critical window for management intervention. Control measures applied before 6 to 8 weeks after emergence may prevent rhizome development, which is essential because once rhizomes are established, new plants can regenerate even after aboveground foliage is removed. Despite differences in plant growth due to environmental variation as observed in this study, rhizome formation occurred at both sites within 7-14 weeks after seed sowing. This demonstrates the adaptability of knotroot foxtail and its ability to survive under a wide range of conditions. Thus, early intervention before these growth stages is necessary to prevent rhizome development. To further understand rhizome formation timing across different ecotypes, additional populations should be studied across the southeastern United States. Deeper anatomical studies are also needed to determine the exact developmental stage when rhizomes begin forming. Future research should also explore environmental cues that trigger the shift to perennial growth in *S. parviflora* to better inform when control should be applied.

Seedhead formation also varied among populations, occurring approximately 60–67 days after planting. This has implications for seed production and dispersal. Delayed flowering can result in prolonged seed rain and increase the soil seedbank, complicating control efforts. Control strategies such as mowing should be well timed. If new plants continues to emerge, it should be implemented before seedhead development to limit seed production.

Accurate identification of knotroot and yellow foxtail is essential for proper management. Morphological traits such as first true leaf width (wider in yellow foxtail), leaf margin roughness (rougher in yellow foxtail), internode length (longer in yellow foxtail), leaf color (blueish green in knotroot and green in yellow foxtail) could aid in distinguishing these species when they are young. Other traits such as spikelet width, seed size, and leaf color are usually required when plants are matured. However, no single trait is sufficient in identifying these two foxtail species, rather, combinations of traits might offer more reliable identification. Leaf color (blueish green in knotroot and green in yellow foxtail) is a useful trait, but subjectivity in color perception may limit its usefulness in distinguishing between the two foxtails. Future studies could explore digital tools or imaging technologies to enhance accuracy in species identification.

In practice, regular field scouting for early identification and timely management are critical in managing yellow and knotroot foxtail in pastures and hayfields. Targeted herbicide applications, mowing, mechanical removal, or grazing should be timed before rhizomes form to minimize both vegetative and reproductive spread. In addition, adopting practices that promote the growth of desirable forage species such as proper fertilization, irrigation, and grazing management can help these forages outcompete knotroot and yellow foxtail. Ensuring early management intervention can prevent knotroot foxtail from becoming a persistent problem and reduce its impact on forage productivity.

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List of Figures and Tables

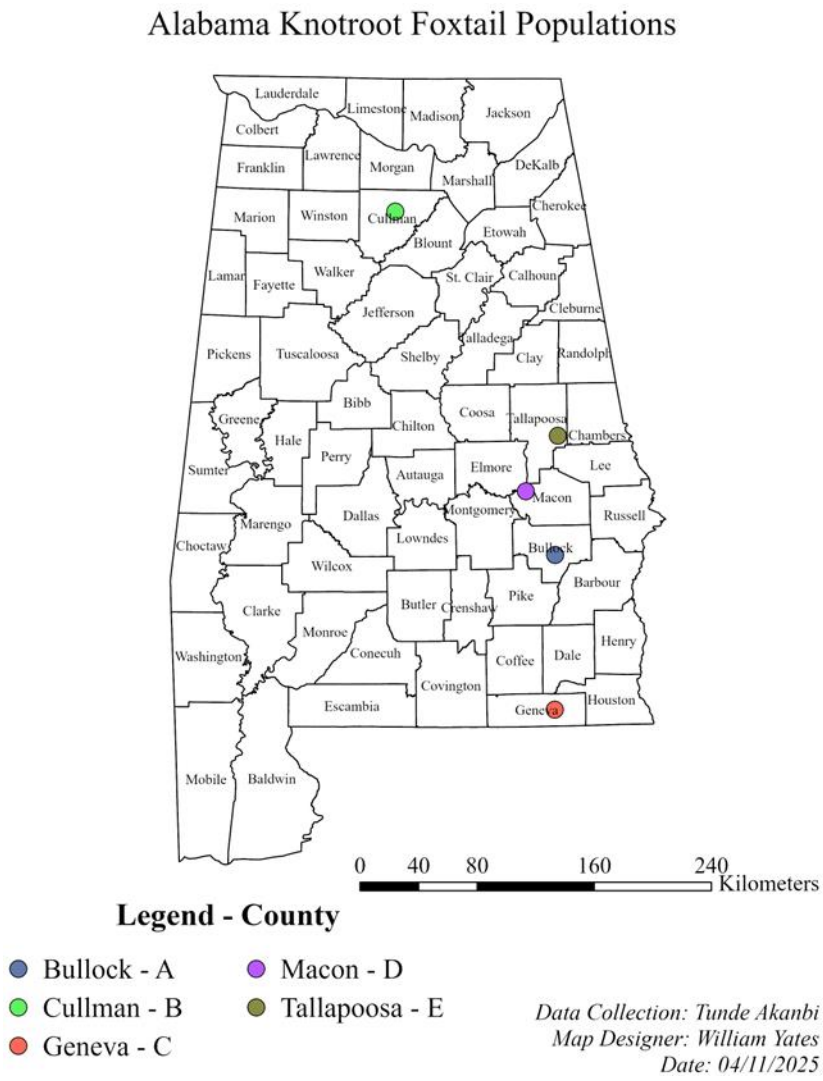

Figure 2-1: Map showing the Collection Sites for Knotroot Foxtail Seeds

Table 2-1: Physio-chemical properties of soil samples at the two locations (Turf and Beef Unit)

Soil Properties	Soil sample (Turf unit)	Soil sample (Beef unit)
pH	6.2	6.6
Phosphorus (lb/A)	37 (M)	227 (VH)
Potassium (lb/A)	23 (L)	109 (M)
Magnesium (lb/A)	85 (H)	685 (H)
Calcium (lb/A)	789 (H)	4284 (H)
Nitrogen (lb/A)	60.0	60.0
P_2O_5	40.0	0.0
K_2O	60.0	40.0
CEC $cmol_c kg^{-1}$	4.6	> 9.0
Soil group	Sandy soil	Clay

L- Low, M-Medium, H-High, VH-Very High

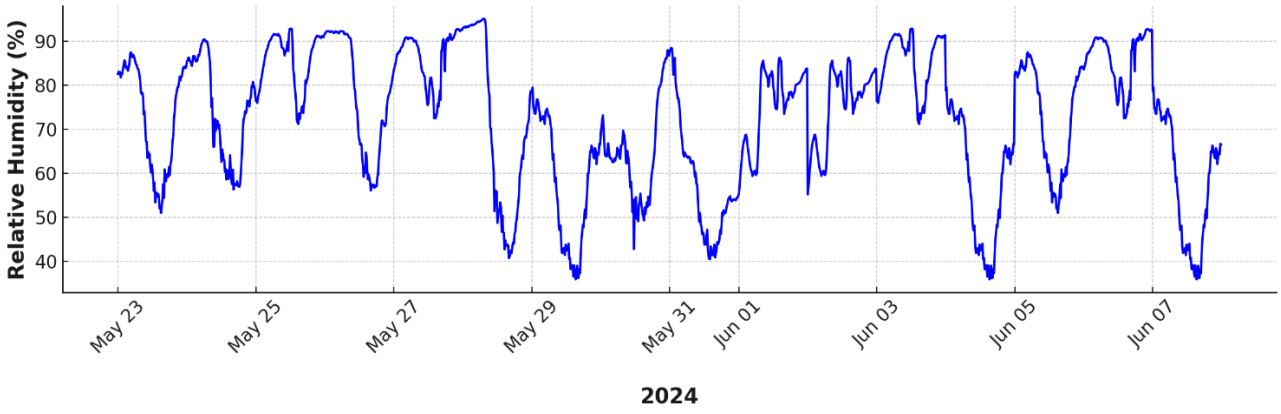

Figure 2-2: Daily relative humidity (%) recorded at the Plant Science Greenhouse, Auburn University, during seedling emergence of five knotroot foxtail populations from May 23 to June 7, 2024.

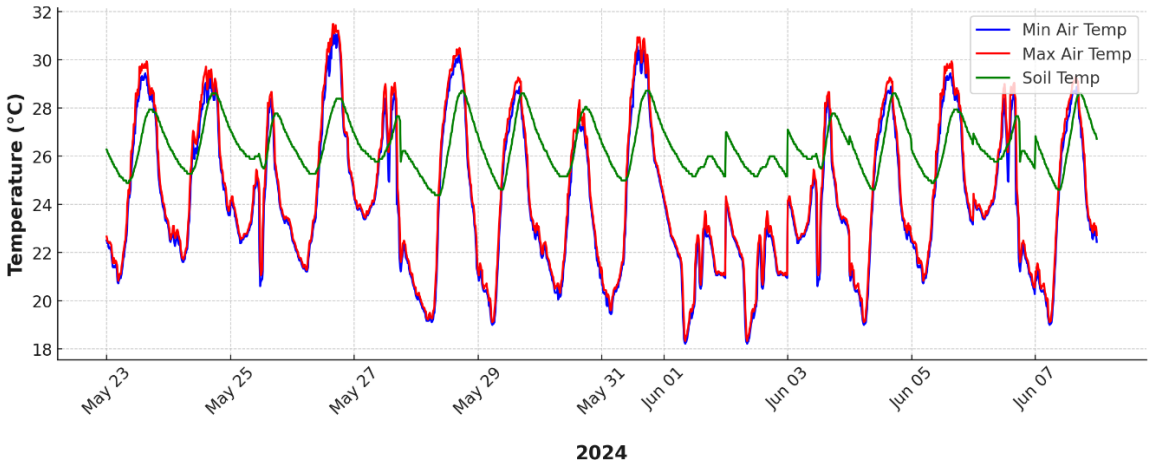

Figure 2-3: Daily minimum and maximum air temperatures, along with daily soil temperature (°C), recorded at the Plant Science Greenhouse, Auburn University, during seedling emergence of five knotroot foxtail populations from May 23 to June 7, 2024.

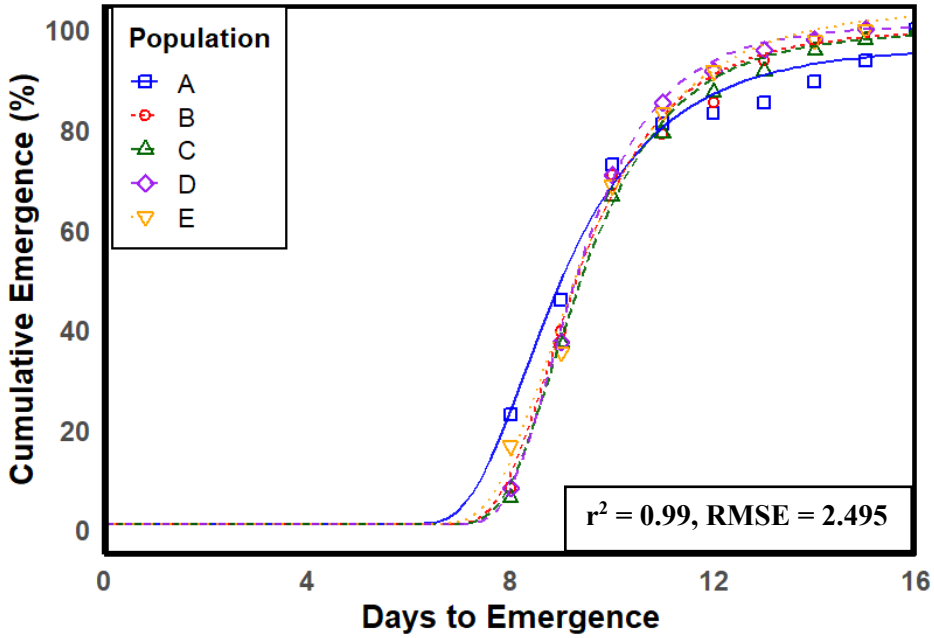

Figure 2-4: Cumulative seedling emergence (%) of knotroot foxtail over time (days after sowing) for five populations, modeled using a Weibull distribution.

Table 2-2: Regression parameter estimates with their standard errors in parentheses of cumulative emergence among the 5 knotroot foxtail populations as estimated with the Weibull type I model.

Population	**Relative Slope (Std. Error)**	**Max (Std.Error)**	**E_{50} (Days)**	**E_{95} (Days)**
A	-6.45 (0.78)	95.83 (2.49)	8.96 b	13.43 b
B	-7.84 (0.86)	99.08 (2.62)	9.32 a	12.99 a
C	-7.91 (0.80)	98.74 (2.16)	9.41 a	13.08 b
D	-8.80 (0.93)	100.00 (2.25)	9.31 a	12.52 a
E	-6.92 (0.72)	100.00 (2.99)	9.39a	13.67 b

Note: E_{50}, E_{95} = number of days required for 50% and 95% germination to occur in each population, Std.Error = Standard Error, Max = Maximum emergence (Upper asymptote). Estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$

Table 2-3: Regression parameter estimates with standard errors in parentheses for time to rhizome formation among the 5 knotroot foxtail populations as estimated with the Weibull type I model.

Population	Beef Unit						Turf Unit					
	b	***d***	**RF_{50}**	**RF_{95}**	**R^2**	**RMSE**	***b***	***d***	**RF_{50}**	**RF_{95}**	**R^2**	**RMSE**
A	5.07 (2.03)	1.00	8.10 a	10.81 b	0.65	0.27	19.64 (3.93)	1.00	7.69 a	7.84 a	0.81	0.20
B	2.91 (1.11)	1.00	7.93 a	13.11 ab	0.50	0.33	3.90 (1.37)	1.00	8.02 a	11.67 ab	0.58	0.29
C	7.98 (3.12)	1.00	6.56 a	6.65 a	0.60	0.27	31.70 (1.93)	1.00	6.96 a	7.29 a	0.54	0.31
D	5.99 (2.34)	1.00	9.67 a	9.89 b	0.82	0.20	3.51 (1.39)	1.00	8.77 a	13.31 ab	0.50	0.33
E	5.69 (2.22)	1.00	6.68 a	6.84 a	0.49	0.31	39.46 (3.93)	1.00	8.97 a	9.31 a	0.76	0.24

b = relative slope, d = Maximum rhizome formation (Upper asymptote), RF_{50}, RF_{95} = number of weeks required for 50% and 95% rhizome formation between the population, respectively. RMSE = Root Mean Square Error. R^2= coefficient of determination, estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$. Lower and upper limits fixed at 0 and 1.

Table 2-4: Regression parameter estimates with their standard errors in parentheses for the relationship between rhizome formation and plant height among the 5 knotroot foxtail populations estimated with the three parameter log-logistic model

Population	Beef Unit					Turf Unit				
	b	***d***	**RF_{50}**	**RF_{95}**	**R^2**	***b***	***d***	**RF_{50}**	**RF_{95}**	**R^2**
A	- 2.5 (1.0)	1.0	17.8 a	58.1 a	0.5	-7.2 (3.5)	1.0	48.6 a	73.3 a	0.7
B	-2.1 (0.8)	1.0	16.7 a	66.2 a	0.4	-4.4 (1.6)	1.0	49.9 a	98.1 ab	0.4
C	-3.7 (2.1)	1.0	12.7 a	53.4 a	0.6	-6.3 (2.6)	1.0	40.6 a	64.6 a	0.5
D	-3.8 (1.7)	1.0	24.1 b	52.7 a	0.6	-3.9 (1.4)	1.0	52.6 a	111.6 ab	0.5
E	-2.2 (0.9)	1.0	16.0 a	59.6 a	0.4	-9.9 (3.8)	1.0	52.2 a	70.8 a	0.7

b = relative slope, d = Maximum rhizome formation (upper asymptote), RF_{50}, RF_{95} = plant height at which 50% and 95% of the knotroot foxtail formed rhizome. R^2= coefficient of determination. Estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$. Lower and upper limits fixed at 0 and 1 respectively.

Table 2-5: Regression parameter estimates with their standard errors in parentheses for the relationship between rhizome formation and leaf stage among the 5 knotroot foxtail populations estimated with the three parameter log-logistic model

Population	Beef Unit					Turf Unit				
	b	***d***	**RF_{50}**	**RF_{95}**	**R^2**	***b***	***d***	**RF_{50}**	**RF_{95}**	**R^2**
A	-2.7 (1.1)	1.0	1.2 a	3.6 a	0.3	-5.8 (2.8)	1.0	4.3b	7.0 a	0.7
B	-2.2 (0.8)	1.0	1.4 a	5.5 b	0.4	-2.8 (1.2)	1.0	3.3 a	9.5 b	0.4
C	-2.5 (1.3)	1.0	1.3 a	4.3 b	0.3	-2.9 (1.4)	1.0	2.7 a	7.6 a	0.3
D	-4.7 (2.7)	1.0	1.8 a	3.4 a	0.6	-2.6 (0.9)	1.0	3.5 a	10.9 b	0.4
E	-4.1 (2.1)	1.0	2.3 a	4.8 b	0.4	-7.9 (3.5)	1.0	5.3 b	7.7 a	0.6

b = relative slope, d = Maximum rhizome formation (upper asymptote), RF_{50}, RF_{95} = leaf stage at which 50% and 95% of the knotroot foxtail formed rhizome. R^2= coefficient of determination. Estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$. Lower and upper limits fixed at 0 and 1 respectively.

Table 2-6: Regression parameter estimates with their standard errors in parentheses for the relationship between rhizome formation and root length (cm) among the 5 knotroot foxtail populations estimated with the three parameter log-logistic model

Population	Beef Unit					Turf Unit				
	b	***d***	**RF_{50}**	**RF_{95}**	**R^2**	***b***	***d***	**RF_{50}**	**RF_{95}**	**R^2**
A	- 3.7 (2.1)	1.00	6.7 a	14.8 a	0.2	-2.8 (1.3)	1.0	7.7 a	21.9 a	0.4
B	-3.9 (2.1)	1.00	6.4a	13.4 a	0.3	-3.6 (2.8)	1.0	12.3 b	27.5 a	0.3
C	-2.0 (1.5)	1.00	4.4 a	19.7 a	0.8	-2.2 (1.1)	1.0	7.7 a	30.1 a	0.2
D	-2.9 (1.4)	1.00	7.6 b	20.5 a	0.4	-3.9 (1.8)	1.0	10.5 b	22.4 a	0.5
E	-1.0 (1.6)	1.00	3.9 a	13.2 a	0.3	-2.1 (1.1)	1.0	9.8 a	38.7 b	0.3

b = relative slope, d = Maximum rhizome formation (upper asymptote), RF_{50}, RF_{95} = root length at which 50% and 95% of the knotroot foxtail formed rhizome. R^2= coefficient of determination. Estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$. Lower and upper limits fixed at 0 and 1 respectively.

Table 2-7: Regression parameter estimates with their standard errors in parentheses for the relationship between rhizome formation and fresh rhizome-root biomass (g) among the 5 knotroot foxtail populations estimated with the three parameter log-logistic model

Population	Beef Unit					Turf Unit				
	b	***d***	**RF_{50}**	**RF_{95}**	**R^2**	***b***	***d***	**RF_{50}**	**RF_{95}**	**R^2**
A	- 7.9 (2.9)	1.00	26.4 b	38.2 ab	0.6	-11.4 (4.8)	1.00	39.6 b	51.4 b	0.4
B	-6.8 (2.4)	1.00	31.5 b	48.6 b	0.4	-9.2 (4.4)	1.00	44.3 b	61.1 ab	0.3
C	-5.9 (2.3)	1.00	13.5 a	22.2 a	0.5	-12.3 (6.3)	1.00	21.0 a	26.7 a	0.4
D	-4.5 (2.7)	1.00	11.0 a	20.8 a	0.6	-8.1 (4.2)	1.00	38.4 b	55.3 b	0.5
E	-4.6 (2.4)	1.00	25.7 b	49.0 b	0.4	-11.3 (7.7)	1.00	17.5 a	21.7 a	0.7

b = relative slope, d = Maximum rhizome formation (Upper asymptote), RF_{50}, RF_{95} = Fresh rhizome-root biomass at which 50% and 95% of the knotroot foxtail formed rhizome. R^2= coefficient of determination. Estimates with the same alphabets are not significantly different from each other, $\alpha = 0.05$. Lower and upper limits fixed at 0 and 1 respectively.

Table 2-8: Result of Analysis of Variance table for time to seedhead formation among knotroot foxtail population.

Source of Variation	df	Sum of square	F-value	Pr (>F)
Population	4	733.36	47.85	< 2.2e-16 ***
Residuals	95	364.00		

*, **, *** = significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively, df = degree of freedom**.** Analyses were conducted to test if days to flowering differs between each population

Table 2-9: Mean values of days to seedhead formation among different knotroot foxtail populations

Populations	Days to seedhead
A	59.8 d
B	63.0 c
C	66.8 a
D	60.1 d
E	64.9 b

Mean values with the same alphabet are not significantly different from each other, $\alpha = 0.05$

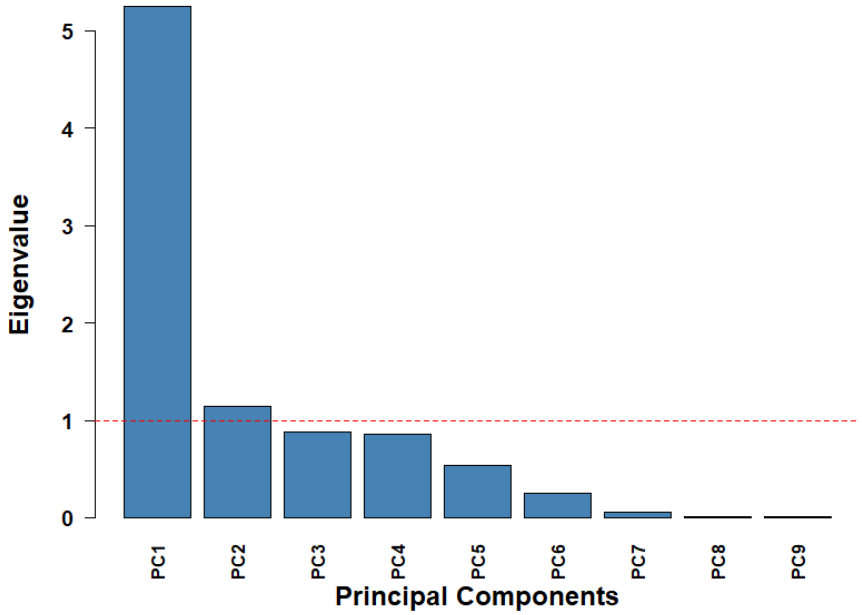

Figure 2-5: Kaiser criterion scree plot, showing the eigenvalues of each principal component (PC) derived from the morphological differentiation between knotroot and yellow foxtail. The red dashed line indicates the eigenvalue threshold of 1, used to determine the number of principal components retained for analysis.

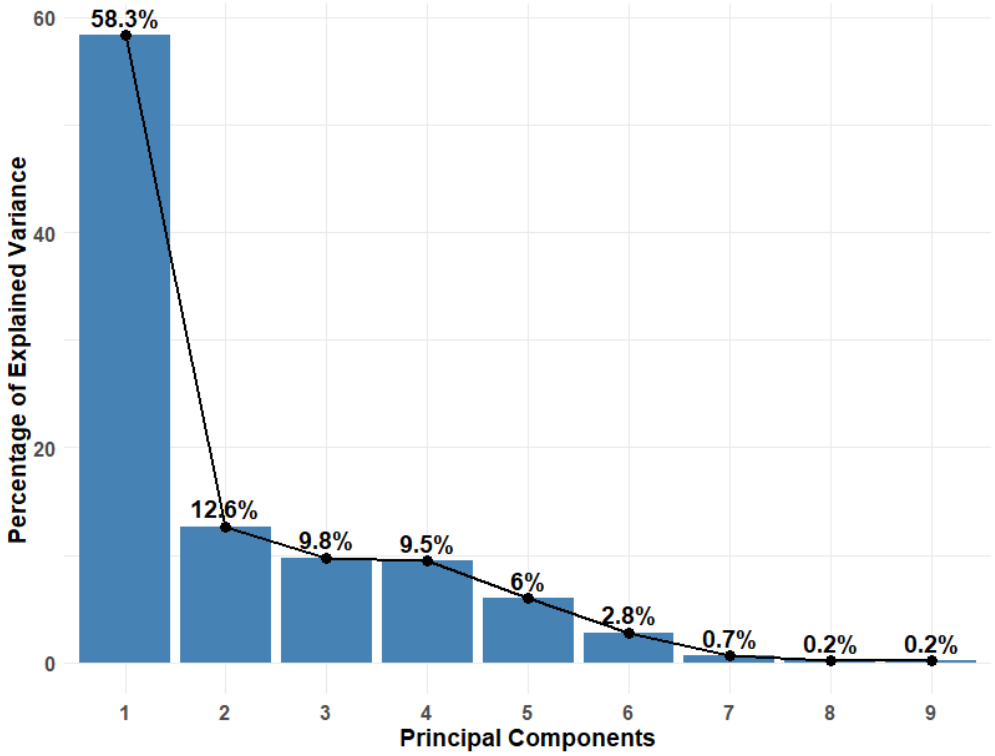

Figure 2-6: Scree plot showing the proportion of morphological variation between yellow and knotroot foxtail explained by each principal component (PC). The percentage labels above each bar indicate the amount of variance accounted for by the corresponding PC, with the first few components capturing the majority of the total variation.

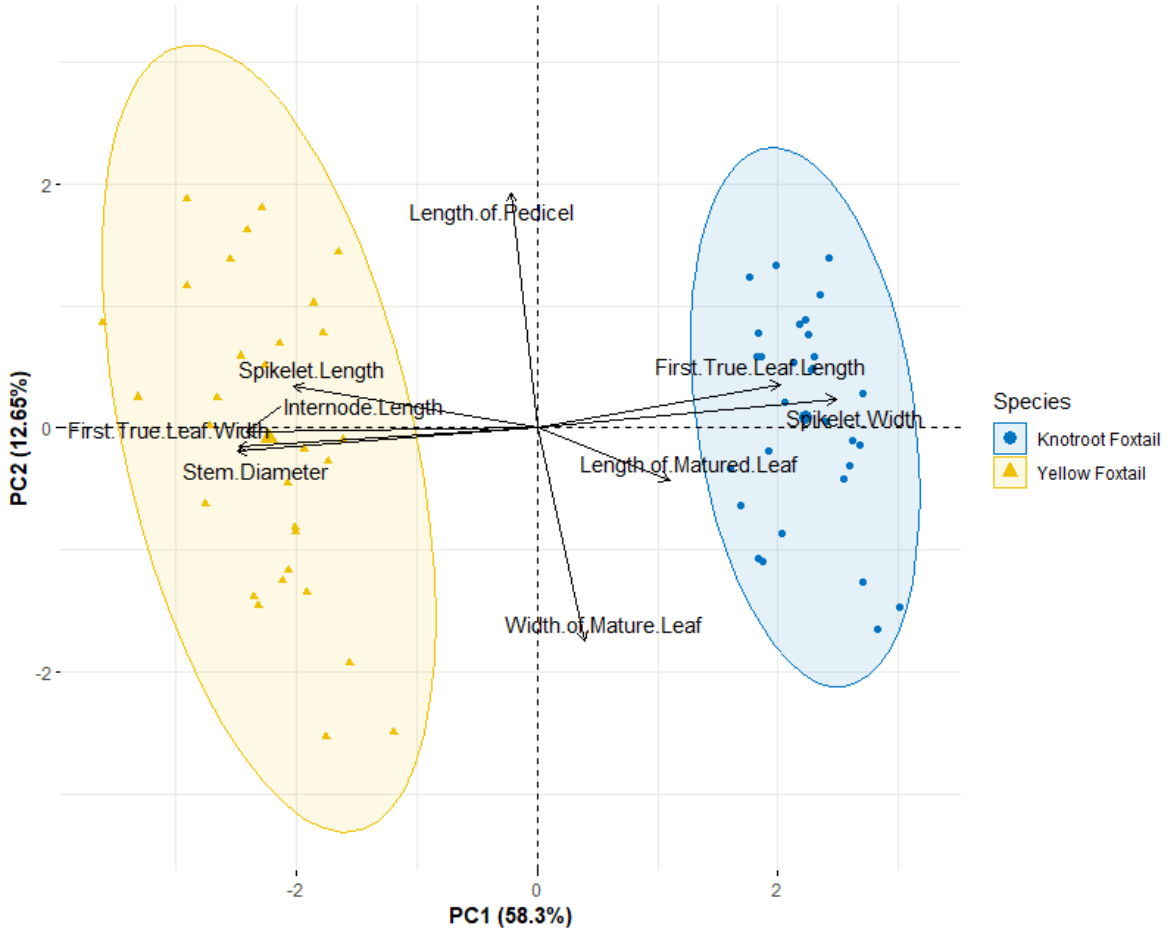

Figure 2-7: Principal Component Analysis (PCA) biplot showing morphological differentiation between knotroot foxtail and yellow foxtail based on nine traits. Each point represents an individual plant, with yellow points and shading for yellow foxtail and blue point and shading for knotroot foxtail. The shaded ellipses highlight the clustering of individuals within each species. PC1 accounts for most of the variation and clearly separates the two species. Arrows indicate the direction and strength of each trait's influence on species separation along the principal components.

Table 2-10. Trait Loadings on the First Two Principal Components and their Contributions to PC1 and PC2

Trait	**PC1**	**PC 2**
Length of first true leaf	0.347	0.134
Width of first true leaf	-0.426	-0.056
Width of Mature Leaf	0.068	-0.647
Length of Matured Leaf	0.190	-0.158
Internode Length	-0.415	-0.012
Stem Diameter	-0.427	-0.072
Spikelet Width	0.426	0.088
Spikelet Length	-0.347	0.124
Length of Pedicel	-0.037	0.713

PC = principal components, Trait loadings indicate the contribution of each morphological variable to the corresponding principal component. positive values suggest the trait is positively associated with the component, while negative values indicate an inverse relationship. Traits with high absolute values (closer to 1 or –1) contribute more strongly to the variation explained by the component.

Table 2-11: Descriptive statistics for distinguishing morphometric traits between knotroot and yellow foxtail identified by the PCA

Morphometric traits	Minimum and Maximum Value		Mean	
	knotroot foxtail	yellow foxtail	knotroot foxtail	yellow foxtail
Length of first true leaf	4.54 - 5.57 mm	3.53 - 4.95 mm	5.05 mm	4.16 mm
Width of first true leaf	0.52 - 1.15 mm	3.00 – 3.69 mm	0.84 mm	3.34 mm
Internode length	2.59 - 5.24 cm	9.73 – 17.92 cm	4.02 cm	14.68 cm
Stem diameter	1.85 - 2.4 cm	4.57 - 4.85 cm	2.09 cm	4.71 cm
Spikelet width	22.05 - 26.95 mm	9.72 – 10.76 mm	24.91 mm	10.24 mm
Spikelet length	54.7 - 71.88 mm	67.78 – 107.73 mm	63.40 mm	85.99 mm

Figure 2-8: First true leaf width of knotroot (A) and yellow foxtail (B)

Figure 2-9: Yellow foxtail (left) and knotroot foxtail seedheads (right) from different populations in Alabama

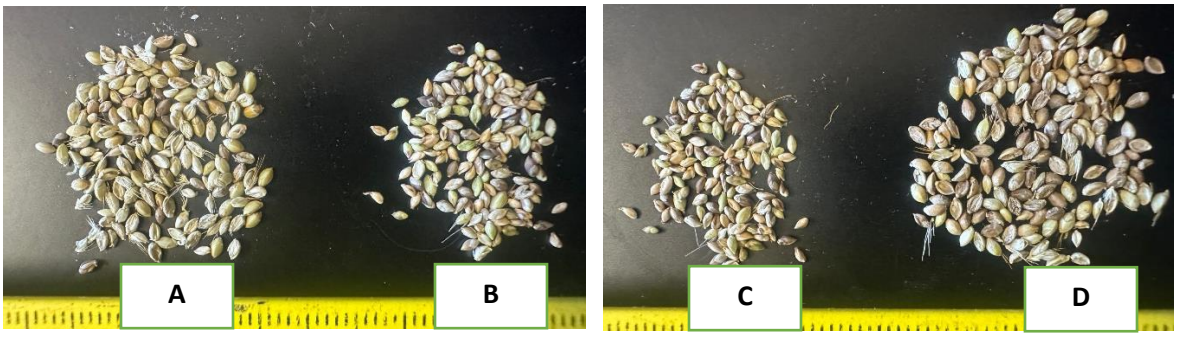

Figure 2-10: Yellow foxtail (**A** and **D**) and knotroot foxtail (**B** and **C**) seeds from different populations.

Figure 2-11a: Upright (**A)** and decumbent (**B)** growing habit of yellow foxtail (*Setaria pumila*) in a hayfield

Figure 2-11b: Upright (**A)** and decumbent **B** growing habit of knotroot foxtail (*Setaria parvilora*) in a pasture field

Figure 2-12: Yellow bristles (awn) on the seedhead of knotroot foxtail (*Setaria parvilora*)

Figure 2-13: Variation in root length and rhizome-biomass from different knotroot foxtail populations collected at the Beef (A) and Turf unit (B).

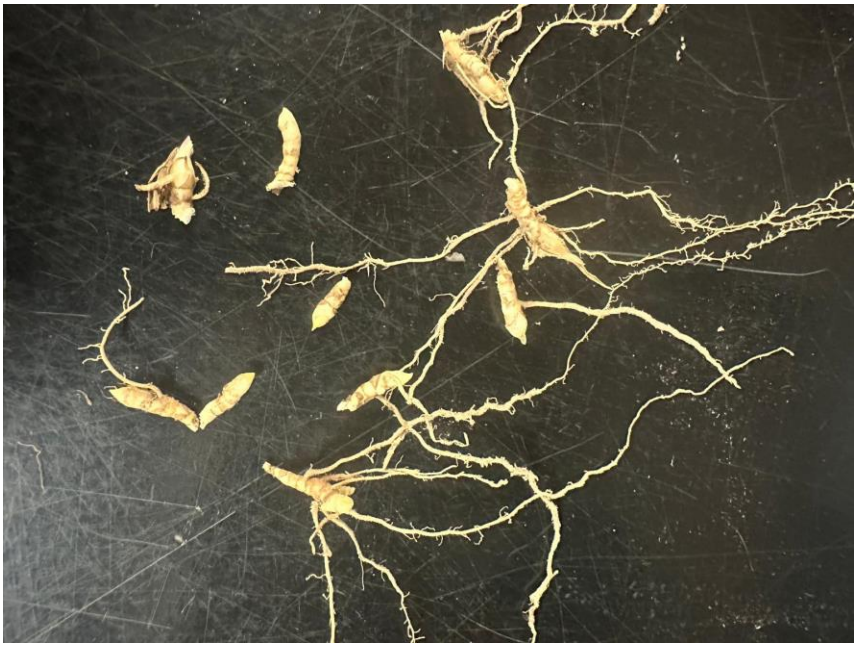

Figure 2-14: Different sizes of knotroot foxtail rhizomes from different population

Chapter III.

Influence of Simulated Rainfall Timing on the Efficacy of Hexazinone and Quinclorac for the Control of Knotroot Foxtail (*Setaria parviflora* (Poir.) Kerguélen).

Abstract

Knotroot foxtail is a perennial grass found in pastures across the Southeastern US. Herbicide active ingredients, hexazinone and quinclorac have shown efficacy in controlling this weed, but their performance is influenced by soil moisture and rainfall activation. Knowledge of proper application before rainfall is essential for maximizing control and to manage for desirable forage species such as bermudagrass, bahiagrass, and tall fescue. Controlled greenhouse study was conducted at Auburn University in 2023 and 2024 to evaluate the response of knotroot foxtail to these herbicides under varied rainfall timings. Knotroot foxtail rhizomes were transplanted into pots and treated once foliage reached 28 cm with quinclorac (0.42 kg ae ha^{-1}) or hexazinone (0.85 kg ai ha^{-1}). Overhead irrigation was calibrated to simulate 0.63 cm (0.25 in) of rainfall 0, 3, 6, 9, 12, and 15 days after herbicide treatment (DAT). Knotroot foxtail control was visually estimated based on injury symptoms at 7, 14, and 51 days after each rainfall timing, and rhizome dry weight biomass was measured at 51 days after treatment (DAT). In 2023, preliminary findings indicated that knotroot foxtail treated with hexazinone had lower rhizome dry weight (1.9 g) compared to those treated with quinclorac (3.2 g), although a non-treated control was not included in this initial evaluation. Similarly, in 2024, with the inclusion of a non-treated control, rhizome dry weight was lowest in hexazinone-treated knotroot foxtail (1.0 g), followed by quinclorac-treated plants (2.1

g), while the non-treated control had the highest value (3.6 g). Across both years, hexazinone consistently provided greater knotroot foxtail control than quinclorac. At 51 days after rainfall treatment (DART), hexazinone resulted in 93% control compared to 82% for quinclorac in 2023, while in 2024, control was 94% for hexazinone and 79% for quinclorac.

In 2023, no significant interaction between rainfall timing and herbicide treatment was observed at 7, 14, and 51 days after treatment for knotroot foxtail control. This lack of interaction may be attributed to transplant shock or drought stress affecting plant responses. In 2024, a significant interaction between rainfall timing and herbicide treatment was observed for knotroot foxtail control at 14 and 51 DAT. At 51 days after rainfall treatment, control with hexazinone ranged from 99% to 92% when rainfall occurred within 0–6 days but declined to 85%–81% when rainfall was delayed until 12–15 days. Similarly, quinclorac treated plants showed 87%–77% control when rainfall occurred within 0–9 days, but control dropped to 67%–62% with delayed rainfall at 12–15 days. Early rainfall (0–6 days) after application ensured optimal performance of both hexazinone and quinclorac, while delayed rainfall (12–15 days) reduced their effectiveness in controlling knotroot foxtail. Planning herbicide application with the knowledge of when rain is expected is crucial to ensure the activation of these herbicides to maximize herbicide performance and prevent poor weed control from delayed rainfall.

Keywords: Efficacy, Forage, Grazing, Knotroot, Rhizome.

Nomenclature: *Setaria parviflora* (Poir.) Kerguelen, hexazinone, quinclorac

Introduction

Knotroot foxtail is a summer perennial grass weed that significantly reduces forage quality and yield in the southeastern United States (Israel *et al*., 2014). Among the various foxtail species (Setaria spp.), knotroot foxtail is also widely distributed across pastures, forage fields, roadsides, Turfs and rangelands throughout the Southeast (Dekker, 2003). It reproduces through seeds and knotty rhizomes formed under its root structure. The presence of the rhizomes makes knotroot foxtail difficult to control as new plants might regenerate even after management efforts and this makes its management different from other annual foxtail species (McCullough, 2016).

To effectively manage this weed in pastures and hayfields, it is crucial to employ an integrated approach combining preventative, cultural, mechanical and chemical control practices. Among these, herbicide application remains a common management strategy to control rhizomatous grass weeds, particularly in grass crops (Grichar and Foster, 2019). However, managing knotroot foxtail in forage systems poses unique challenges due to the limited availability of herbicides that are both effective and safe for use on a wide range of desirable forage grass species (Israel *et al*., 2014). While preemergence herbicide applications in spring with the right environmental condition can control foxtail seedlings from seeds shed in the previous season, new plants emerging from rhizomes of established plants often require postemergence herbicide applications (Dyer *et al*., 2024). The diversity of forage systems in Southeast, United States, further complicates herbicide selection, as no single postemergence herbicide is universally effective and safe across all forage systems. Many available herbicide options may cause crop injury, such as stunting or suppression in bermudagrass or tall fescue (James *et al*., 2009; Blair Griffin and Johnny Gunsaulis, 2019).

Research by Russell *et al*. (2022) identified herbicide active ingredients such as hexazinone and quinclorac as promising herbicides for controlling knotroot foxtail. Hexazinone is safe for use on bahiagrass and bermudagrass, as it causes minimal foliar injury when applied postemergence. Similarly, quinclorac, a synthetic auxin herbicide with selective grass activity, is an effective postemergence option in tall fescue forage. These two herbicides also have both foliar and soil activity, however, their performance is influenced by soil moisture and rainfall activation (William *et al*., 2004).

The fate and efficacy of herbicides depend not only on its chemical properties but also on how it interacts with environmental variables such as rainfall, soil moisture, and plant surfaces after application (Baker *et al*., 1991). Rainfall shortly after or delayed from herbicide application can either enhance or reduce its effectiveness by influencing absorption, translocation, or soil persistence. Understanding how rainfall intervals impact herbicide performance is crucial. Studies by Upchurch *et al*. (1969); Doran and Andersen (1975) reported reduced effectiveness of postemergence herbicides when rain occurs too soon after application. Conversely, Miller and Norsworthy (2018) found that adequate soil moisture can enhance herbicide absorption, particularly for systemic herbicides requiring translocation within the plant. Rainfall occurring after treatment affects the efficacy of postemergence herbicides, and the required rain-free period (rainfastness) for optimal control varies significantly across plant species, herbicide formulations, and application rates (Weaver, 1945; Bryson, 1987). Since hexazinone and quinclorac have both soil and foliar activity, it is important that rainfall is received soon after they are applied. Based on the specific herbicide label recommendation, hexazinone requires about ¼ to ½ inch of rainfall after application for proper soil activation (Tide International USA, 2019). For quinclorac, the label specifies that rainfall or irrigation is needed to move the herbicide into the soil but does not

clearly state the exact amount required (BASF, 2019). Although both herbicides work best under moist soil conditions, there is still a gap in the literature regarding the optimal timing of rainfall after application for effective control particularly for managing knotroot foxtail in forage grass systems. Understanding this timing is critical for improving herbicide performance and reducing the likelihood of herbicide loss due to runoff, leaching, or degradation before effective weed uptake. Proper application strategies that consider rainfall timing are especially important for managing grass weeds in desirable forage species such as bermudagrass, bahiagrass, and tall fescue. Therefore, the objective of this study is to investigate the influence of rainfall timing and herbicide selection following hexazinone and quinclorac application to achieve maximum control of knotroot foxtail.

Materials and Methods

Source of Plant Materials and Greenhouse Experiment Setup

Greenhouse experiments were conducted in 2023 and 2024 to evaluate the response of knotroot foxtail to herbicide application under varying rainfall timings. Mature knotroot foxtail plants were collected from pastures and roadsides near Auburn, Alabama (32.549450N, 85.563370W; 32.445973, -85.900157). The plants were clipped to remove foliage, leaving 12.7 cm (5-inch) stubbles along with intact rhizome and root structures. The stubble from each plant was weighed and transplanted into green 6-inch greenhouse pots filled with sandy loam soil (75% sand, 10% silt, 15% clay). The plants were irrigated adequately and allowed to establish naturally without fertilizer application. To prevent insect damage, malathion insecticide was applied weekly. The greenhouse environment was maintained at day and night temperatures of 33°C ±3°C (91.4°F) and 25°C ±3°C (91.4°F), respectively, with natural light supplemented by sodium vapor lamps to provide a 14-hour photoperiod. The average relative humidity (RH) was maintained at 40%.

Herbicide Treatments and Rates

When the plants attained an average height of 28.5 cm, they were separated into two groups with 48 plants in each herbicide treatment group. The herbicides applied were quinclorac at 0.41 kg ae ha^{-1} (1 qt/A Facet L), hexazinone at 0.84 kg ai ha^{-1} (3 pt/A Tide Hexazinone). Herbicide applications were conducted outside the greenhouse using a CO_2 pressurized backpack sprayer calibrated to deliver 140.3 L ha^{-1} through a single Turbo Teejet TT11002-VP nozzle. Following herbicide treatment, the plants were returned to the greenhouse.

Rainfall Treatments

After herbicide treatment, the treated plants were further subdivided into six groups based on rainfall timing. Each rainfall timing group contained 16 plants (8 treated with quinclorac and 8 treated with hexazinone). A simulated rainfall of 0.63 cm (0.25 inches) was applied using overhead irrigation at six intervals: 0, 3, 6, 9, 12, and 15 days after herbicide treatment. In order to keep the plants alive between rainfall events, individual pots were sub-irrigated by placing a 10 inches clear plastic plant saucer beneath each pot and watered at five-day intervals to maintain soil moisture at field capacity (16% MC) in order to keep the plants alive. The soil moisture levels were monitored using an ML3 ThetaProbe Soil Moisture Sensor.

Experimental Design

The experiment was conducted in a split- plot design with four replicates in both years (2023 and 2024) with four replications. The main plot is the herbicide treatments (quinclorac and hexazinone) and the subplots are the rainfall treatments (0, 3, 6, 9, 12, and 15 days after herbicide treatment). The experiment was laid out such that each herbicide treatment included six different rainfall timing groups. Within each rainfall timing group, there were four replicates, with each replicate consisting of two plants (pots), resulting in a total of 8 plants per rainfall timing group. This setup was the same for both herbicide treatments, leading to a total of 96 plants per experimental run. In 2023, a pilot experimental study was conducted as a single run, while in 2024, the experiment was performed in two independent runs to increase the sample size of the experimental unit. To minimize environmental variation within the greenhouse, especially due to proximity to the cooling panel, all pots were arranged in a completely randomized design and regularly rotated throughout the experiment. This ensured that treatment effects were not confounded by pot position or localized environmental conditions.

Data Collection and Data Analysis

Knotroot foxtail control (%) was visually estimated at 7, 14, and 51 days after initial rainfall treatment based on herbicide injury symptoms, where 0% indicated no visible injury (no control) and 100% represented complete plant death (total control). At 51 days after herbicide treatment in 2023 and 2024, the foliage of the treated plants were cut and the rhizome from these plants were weighed to obtain fresh biomass. The rhizomes were then oven-dried at a constant temperature of 60°C for 3 days to determine the biomass reduction. The biomass was then weighed and the dry biomass of rhizomes were also recorded.

All statistical analysis was performed using the R Studio (version 2023.3.0.386). The data were analyzed using a linear mixed-effects model with herbicide, rainfall timing including their interaction as fixed effects and replicates as random effect as given by the following model:

$$\text{control}_{ijk} = \beta_0 + \beta_1(\text{herbicide}_i) + \beta_2(\text{rainfall timing}_j) + \beta_3(\text{herbicide}_i \times \text{rainfall timing}_j) + \mu_k + \mu_{ki} + \varepsilon_{ijk}$$

Where:

β_0 = Overall intercept

β_1, β_2, β_3 = Fixed effects coefficients for herbicide, rainfall timing, and their interaction

μ_k = Random effect of replication (rep)

μ_{ki} = Random effect of herbicide nested within replication

ε_{ijk} = Residual error for the i^{th} herbicide, j^{th} rainfall timing and k^{th} replicate

During the data analysis, rainfall timing is set as a continuous variable, while herbicide is treated as categorical variable. The data collected from the pilot study in 2023 was analyzed as a single experimental run. In 2024, data from both experimental runs were analyzed using a linear mixed-effects model, and ANOVA was used to test for the significance of the experimental run and its interaction with treatment factors (herbicide and rainfall timing). When no significant interactions were observed between rainfall timing and herbicide treatments on knotroot foxtail control and dry rhizome biomass, Analysis of Variance (ANOVA) was conducted to evaluate the effect of herbicide treatments and compare treatment means across the evaluation periods. Means were separated using Tukey's Honest Significant Difference (HSD) test. Control treatments were excluded from the analysis for knotroot foxtail control, as no visible injury was observed in the non-treated plants. In 2024, a mixed effects linear regression model was fitted to examine the interaction between herbicide and rainfall timing. The relationship between knotroot foxtail control and dry rhizome biomass was assessed using the Spearman's rank correlation coefficient (ρ) where, "0" is no correlation among variables, "1" is positive correlation, and "− 1" is negative correlation, using this following equation,

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}$$

Where, "d" are the differences between the ranks for each pair of observations and "n" is the number of paired values or observations (Spearman, 1904).

Results

Effect of interaction herbicide and rainfall timing on knotroot foxtail control in 2023.

In 2023, the regression analysis revealed that there was no significant interaction between rainfall timing and herbicide application with respect to knotroot foxtail control at 7 ($P = 0.08$), 14 ($P = 0.69$) and 51 ($P = 0.85$) days after each rainfall treatment (Table 1). However, the main effect of herbicide treatment significantly influenced knotroot foxtail control at all three evaluation timings in 2023 ($P < 0.001$). Therefore, control was evaluated based on herbicide treatment alone. Knotroot foxtail plants treated with hexazinone at 0.84 kg ai ha^{-1} consistently exhibited greater control compared to those treated with quinclorac at 0.41 kg ae ha^{-1} in 2023 (Table 1). At 7 days after rainfall treatment, hexazinone resulted in 92% control of knotroot foxtail, while quinclorac provided 82% control. At 14 days after rainfall treatment, hexazinone provided 99% control, compared to 92% with quinclorac. Also, at 51 days after rainfall treatment, control remained higher with hexazinone (93%) than with quinclorac (82%) (Table 1).

Effect of interaction of herbicide and rainfall timing on knotroot foxtail control in 2024.

In 2024, data from both experimental runs were combined when no treatment-by-run interaction was detected for knotroot foxtail control at 51 DAT (herbicide x runs, $P = 0.45$; herbicide x rainfall x runs, $P = 0.55$) and for rhizome dry biomass weight at 51 DAT herbicide treatment (herbicide x runs, $P = 0.83$; herbicide x rainfall x runs, $P = 0.96$). Regression analysis of the pooled data revealed a significant interaction between herbicide treatment and rainfall timing at 14 ($P = 0.006$) and 51 DAT ($P = 0.03$), indicating that herbicide efficacy varied depending on the timing of rainfall following application (Table 2). Therefore, rainfall timing effects were

reported separately for each of the two herbicides (hexazinone and quinclorac) at both 14 and 51 DART.

The efficacy of hexazinone and quinclorac was significantly reduced as rainfall timing after herbicide application increased. At 7 DART, there was no significant interaction between herbicide and rainfall timing. However, by 14 DART, early rainfall (0 to 3 days after application) led to greater knotroot foxtail control with hexazinone (84–83%) compared to delayed rainfall at 12 or 15 days, which reduced control to 79% and 78%, respectively (Figure 1). A similar results were observed for plants treated with quinclorac. Rainfall received immediately after application (0 day) resulted in 69% knotroot foxtail control, whereas delaying rainfall by 15 days reduced control to 55%. At 51 DART, rainfall application same day after hexazinone application resulted in nearly complete control (<99%) followed by rainfall at 3 to 6 days (92–96%). Delaying rainfall to 12 or 15 days reduced control to 85% and 81%, respectively (Figures 2 and 3).

For quinclorac, control gradually declined with increasing delay in rainfall. When rainfall occurred on the same day as herbicide application resulted in higher knotroot foxtail control (87%). However, as rainfall was delayed to 3, 6, and 9 days, knotroot foxtail control levels gradually decreased to 82%, 77%, and 72%, respectively. When rainfall was delayed until 12 and 15 days after quinclorac application, control declined to 67% and 62%, respectively (Figures 2 and 4).

Effect of herbicide on knotroot foxtail control in 2024

Herbicide treatment had a significant effect on knotroot foxtail control at 7, 14, and 51 days after rainfall in 2024 ($P < 0.001$). In 2024 study, hexazinone consistently provided greater control of knotroot foxtail compared to quinclorac across all evaluation periods. At 7 DART, hexazinone provided 68% knotroot foxtail control, compared to 50% with quinclorac (Table 1). At 14 DART,

hexazinone provided 81% knotroot foxtail control, which was greater than the 62% control observed with quinclorac. Similar results were observed at 51 DART, with hexazinone achieving 90% control, compared to 75% for quinclorac (Table 1). Variation in herbicide performance in the study years suggests that the efficacy of herbicide could be influenced by environmental factors such as rainfall timing (Varansi *et al*., 2016).

Effect of herbicide treatment on dry rhizome biomass in 2023 and 2024

The analysis of variance model revealed no significant interaction between herbicide and rainfall timing on dry rhizome biomass in 2023 and 2024 ($P = 0.83$, 0.92, respectively). However, herbicide application, had a significant impact on rhizome biomass ($P < 0.001$). Thus, the effect of herbicide application was presented independently. Plants treated with hexazinone had less dry rhizome biomass compared to plants treated with quinclorac in both study years. In 2023, knotroot foxtail treated with hexazinone recorded a rhizome biomass of 1.88 g, while plants treated with quinclorac had rhizome biomass of 3.24 g (Figure 5).

In 2024, the dry biomass of non-treated plants was collected for each run and included in the data analysis to observe the efficacy of each herbicide treatment on dry rhizome biomass at 51 days after herbicide application. Plants treated with hexazinone recorded the lowest dry rhizome biomass of 1.02 g (72% biomass reduction compared to the non-treated plants) followed by plants treated with quinclorac with dry rhizome biomass of 2.09 g (42% compared to the non-treated control). Non-treated plants recorded the highest rhizome biomass weight of 3.58 g (Figure 6-7).

Correlation between Knotroot foxtail control and dry rhizome biomass

The Spearman rank-based correlation test revealed a significant negative correlation between knotroot foxtail control and dry rhizome biomass. At 51 days after herbicide treatment in

2023, the correlation coefficient was ρ = -0.206 (P = 0.04), indicating that increased control was slightly associated with lower rhizome biomass. A stronger negative correlation (ρ = -0.770, P < 0.001) was observed at 51 days after treatment in 2024, suggesting that higher levels of knotroot foxtail control were strongly associated with a significant reduction in rhizome biomass (Figures 9–10). These findings indicate that effective herbicide applications, resulting in greater control, can substantially reduce rhizome biomass.

Discussion

The variation in knotroot foxtail control in response to herbicides underscores the importance of environmental factors, such as rainfall and soil moisture, in influencing herbicide efficacy performance. However, a study by (Anderson and Arnold, 1985; Weaver *et al*, 1946) found that the efficacy of some post emergence herbicide was reduced when rainfall was received earlier after herbicide application. The variation in these findings could be attributed to differences in herbicide rainfastness (Souza *et al*., 2014), movement within the plant, formulation, and mode of action. It is also important to note that both hexazinone and quinclorac exhibit both soil and foliar activity. This study strongly suggests that soil moisture and rainfall timing play a pivotal role in activating these herbicides, allowing them to translocate to the roots and rhizomes effectively.

In this study, when rainfall was delayed beyond 12 days after herbicide application, regrowth from rhizomes was observed, indicating reduced herbicide efficacy. Although, Weaver *et al*. (1946) proved that an oil-based adjuvant could help systemic herbicides maintain effectiveness and overcome rainfall variability, these are not often recommended with hexazinone as excessive injury to desirable forages are likely to occur. The reduced efficacy of both hexazinone and quinclorac with delayed rainfall suggests that timely rainfall and favorable growing conditions enhances herbicide uptake, likely due to improved activation and root translocation. However, the

degree to which herbicide activity is affected by reduced moisture varies depending on the physicochemical properties of each herbicide.

The lack of significant interaction between herbicide and rainfall timing in 2023 may be attributed to drought or transplant stress, which likely influenced the plants' response to herbicide treatments as every plant did not get enough moisture to keep them alive during the study. However, in the second-year experimental runs in 2024, knotroot foxtail plants were established under optimal conditions, with consistent soil moisture levels maintained at field capacity. These data suggest that improved conditions resulted in significant interaction between rainfall timing and herbicide treatment for knotroot foxtail control at 14 and 51 days after treatment (DART). These findings emphasize the need for a comprehensive understanding of environmental factors, herbicide characteristics, and application timing to optimize weed control strategies for perennial weeds like knotroot foxtail.

Herbicide selection significantly influenced rhizome biomass reduction and knotroot foxtail control. Across both study years, hexazinone (Tide Hexazinone at 3 pt/A) consistently outperformed quinclorac (Facet L at 1 qt/A) in reducing rhizome biomass and improving knotroot foxtail control. Similar result was obtained by Burns (2006) who found that hexazinone at 1.26 kg ai ha^{-1} used alone controlled knotroot foxtail by more than 80% at 4 and 6 wk after application in comparison to other post emergent options for knotroot foxtail control. Coats *et al*. (1998) reported significantly higher knotroot foxtail control with hexazinone, achieving 80–90% control at 2 and 4 months after treatment (MAT) with 13.45 kg ai ha^{-1}. The lower efficacy of quinclorac compared to hexazinone could be linked to differences in herbicide modes of action, herbicide water solubility and soil adsorptive properties as compared to hexazinone. Williams and Walker (2004) reported that the loss of quinclorac efficacy in torpedograss over time may be due to the leaching

of soil-applied quinclorac beyond the rooting zone. They noted that some amount of quinclorac tends to be retained on the leaf surface, without being readily absorbed or translocated within the plant, which may also contribute to reduced efficacy in knotroot foxtail. This underscores the importance of adequate rainfall or irrigation to wash the herbicide off the foliage and reintroduce it into the soil for effective root uptake. A different study by Rector *et al*. (2018) reported that established knotroot foxtail might not controlled by quinclorac because it emerges from a rhizome.

From this study, quinclorac, while less effective compared to hexazinone, still achieved a 41.62% reduction in rhizome biomass, demonstrating its potential as an alternative option in situations where hexazinone is unavailable or unsuitable for use. Additionally, evaluating the residual effects of these herbicides on rhizome regrowth and seed production in subsequent growing seasons could provide valuable insights into their long-term efficacy. It is also important to consider that good growing conditions favor desirable forage species by enhancing their ability to metabolize and detoxify herbicides like quinclorac and hexazinone. This not only reduces the risk of crop injury but also allows for quicker recovery and improved competitiveness against knotroot foxtail following treatment.

The significant negative correlation observed between knotroot foxtail control and rhizome biomass underscores the importance of achieving high levels of control to effectively suppress rhizome development. The observed decline in rhizome biomass across herbicide-treated plots further highlights the potential of herbicides like hexazinone and quinclorac to suppress perennial weeds such as knotroot foxtail through rhizome degradation to prevent regrowth and future infestations. This aligns with the understanding that perennial weeds like knotroot foxtail rely on rhizome reserves for regrowth, and effective herbicide application can disrupt this process. The movement of these two herbicides (hexazinone and quinclorac) from soil and leaves to roots and

rhizomes emphasizes the ability of their potential to effectively target underground structures critical for perennial weed survival. This process could be particularly advantageous in late-season applications when the translocation of herbicides moves with the downward movement of carbohydrates toward rhizomes, as observed in previous studies (Wilson *et al*., 2006; Klingman and Ashton, 1975).

Practical Implication

For effective control of knotroot foxtail in desirable forage systems, hexazinone applied at 0.839 kg ai ha^{-1} (3 pt/A Tide Hexazinone) and quinclorac applied at 0.414 kg ae ha^{-1} (1 qt/A Facet L) could provide considerable suppression of knotroot foxtail especially when they are young preferably before rhizome development. Herbicide selection should be carefully done having in mind the base forage in the field as quinclorac is safe for use on bermudagrass and tall fescue, while hexazinone is suitable for use on bahiagrass and bermudagrass. Integrating these herbicides with other sound cultural practices such as proper fertilization and strategic grazing management can enhance the control of knotroot foxtail in pastures and hayfield and improve overall forage productivity.

Knowledge of forecasted rainfall is also important for herbicide application planning and timing as adequate amount of moisture, at least 0.25cm (1/4 inch) of rainfall, is required to activate these herbicides. This is because early rainfall within 0-9 days after application enhanced the transport of herbicide into the soil or foliage efficiently, leading to better control. The reduction in efficacy is observed when new growth starts to form 51 days after treatment on plants that receive rainfall later than 9 days.

Additionally, producers are encouraged to scout the field to identify the type of foxtail species on the field. This is also important for herbicide selection purposes as misidentification can lead to failure of selected management programs. Knotroot foxtail is the only foxtail commonly found in the southeastern United States roadsides, pasture and hayfields that forms rhizome under its root mass. Given the limited herbicide options available for managing grass within grass systems, future studies should explore additional herbicides with different modes of action to expand weed management strategies and enable effective herbicide rotation.

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List of Tables and Figures

Table 3-1. Type II Wald chi-square test for the effects of herbicide, rainfall timing, and their interaction on knotroot foxtail control at 7, 14, and 51 days after rainfall treatment (DART) in 2023.

Source of variation	(7 DART)		(14 DART)		(51 DART)	
	χ^2	Pr(>Chisq)	χ^2	Pr(>Chisq)	χ^2	Pr(>Chisq)
Herbicide	28.57	< 0.001	30.88	< 0.001	4.86	0.027
Rainfall	19.68	< 0.001	0.68	0.41	2.46	0.12
Herbicide × Rainfall	2.99	0.083	0.16	0.69	0.04	0.85

χ^2 = value of the type II Wald chi square; DART = Days after Rainfall Timing, Pr(>Chisq) = p-value of the type II Wald chi square, $\alpha = 0.05$

Table 3-2. Effect of herbicide treatment on knotroot foxtail control at each evaluation date in 2023 and 2024 greenhouse study.

Herbicide treatment	7 DART (%)		14 DART (%)		51 DART (%)	
	2023	2024	2023	2024	2023	2024
hexazinone (0.84 kg ai ha^{-1})	92.1b	68.3a	98.9b	81.0b	92.5b	90.1b
quinclorac (0.41 kg ae ha^{-1})	81.5a	50.0b	91.7a	62.0a	81.6ba	76.4a

* Different letters denote significant difference, $\alpha = 0.05$, DART = Days After Rainfall Treatment

Table 3-3. Type II Wald chi-square test for the effects of herbicide, rainfall timing, and their interaction on knotroot foxtail control at 7, 14, and 51 days after rainfall treatment (DART) in 2024.

Source of variation	(7 DART)		(14 DART)		(51 DART)	
	χ^2	Pr(>Chisq)	χ^2	Pr(>Chisq)	χ^2	Pr(>Chisq)
Herbicide	180.70	< 0.001	409.21	< 0.001	271.67	< 0.001
Rainfall	67.72	< 0.001	54.04	< 0.001	246.49	< 0.001
Herbicide × Rainfall	1.15	0.28	7.42	0.006	4.58	0.03

χ^2 = value of the type II Wald chi square; DART = Days after Rainfall Timing, Pr(>Chisq) = p-value of the type II Wald chi square, $\alpha = 0.05$

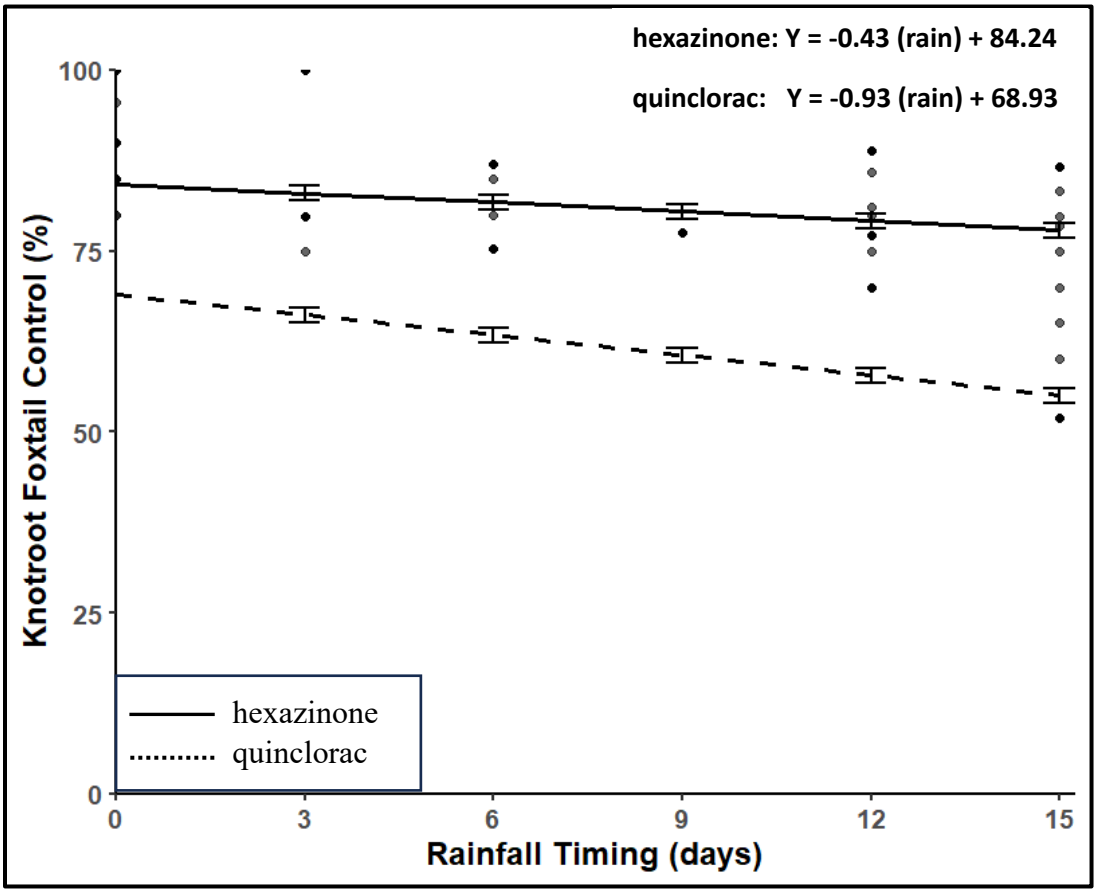

Figure 3-1: Two regression lines for the interaction effect of simulated rainfall timing (days) on the activity of herbicide on knotroot foxtail control at 14 days after rainfall treatment. The regression parameter estimates of the curves and the model performance estimate are shown on Table 4 and Table 6 respectively.

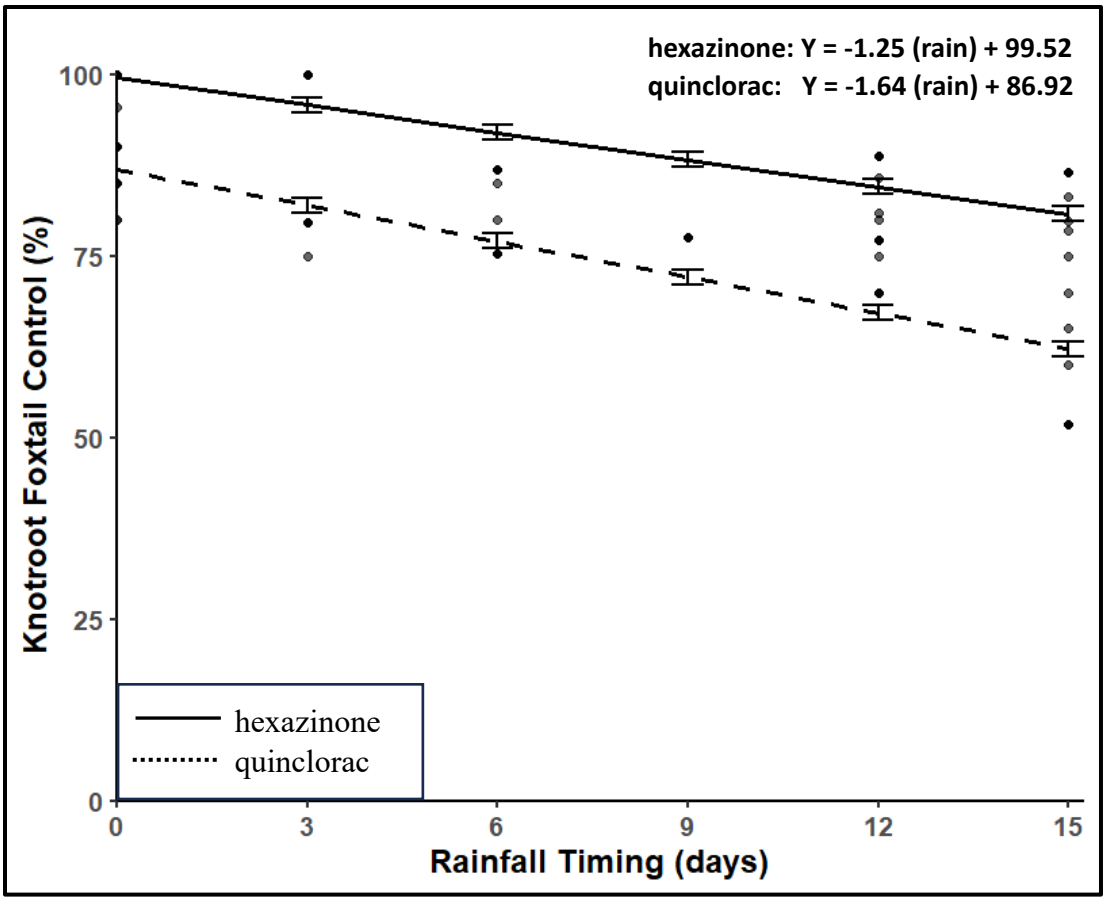

Figure 3-2. Two regression lines for the interaction effect of simulated rainfall timing (days) on the activity of herbicide on knotroot foxtail control at 51 days after rainfall treatment. The regression parameter estimates of the curves and the model performance estimate are shown on Table 5 and Table 6 respectively.

Table 3-4: Model performance parameter for the plot the effect of simulated rainfall timing (days) on the activity of herbicide on knotroot foxtail control at 14 and 51 days after rainfall treatment

Model description	**Marginal R^2**	**Conditional R^2**	**RMSE**
Knotroot foxtail control at 14 DART (Fig 3-1)	0.711	0.712	6.462
Knotroot foxtail control at 51 DART (Fig 3-2)	0.731	0.733	6.483

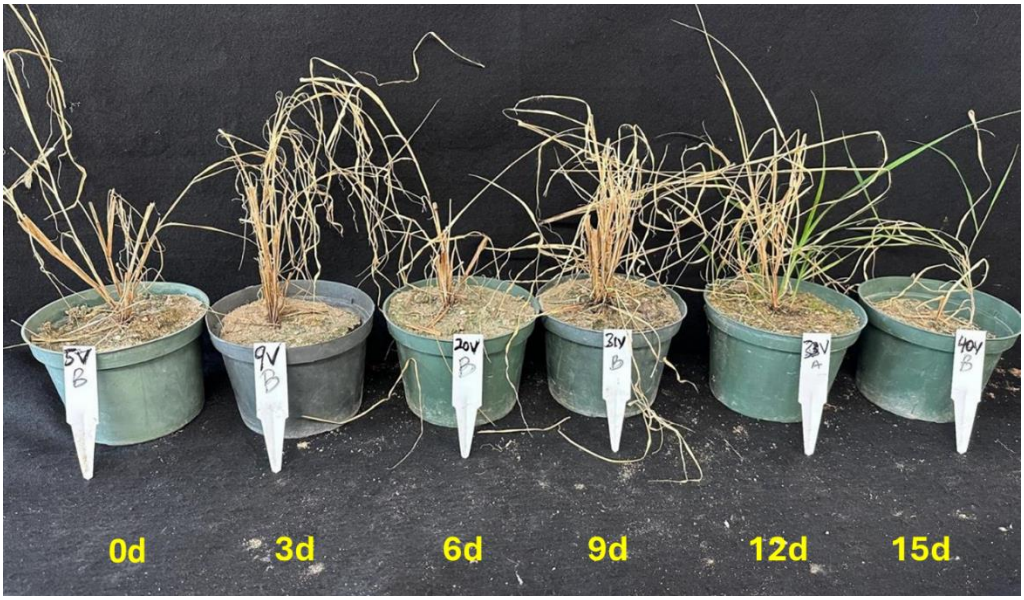

Figure 3-3: Knotroot foxtail treated with 0.839 kg ha^{-1} (3pt/A) hexazinone 51 days after rainfall treatment in 2024, d= days after herbicide treatment

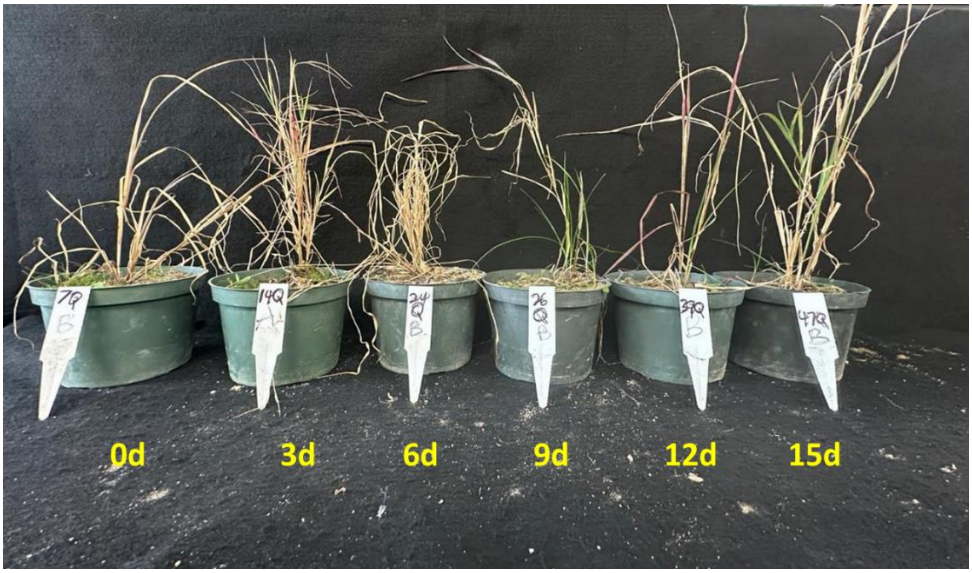

Figure 3-4: Knotroot foxtail treated with 0.414 kg ha^{-1} (1qt/A) quinclorac 51 days after rainfall treatment in 2024, d= days after rainfall treatment

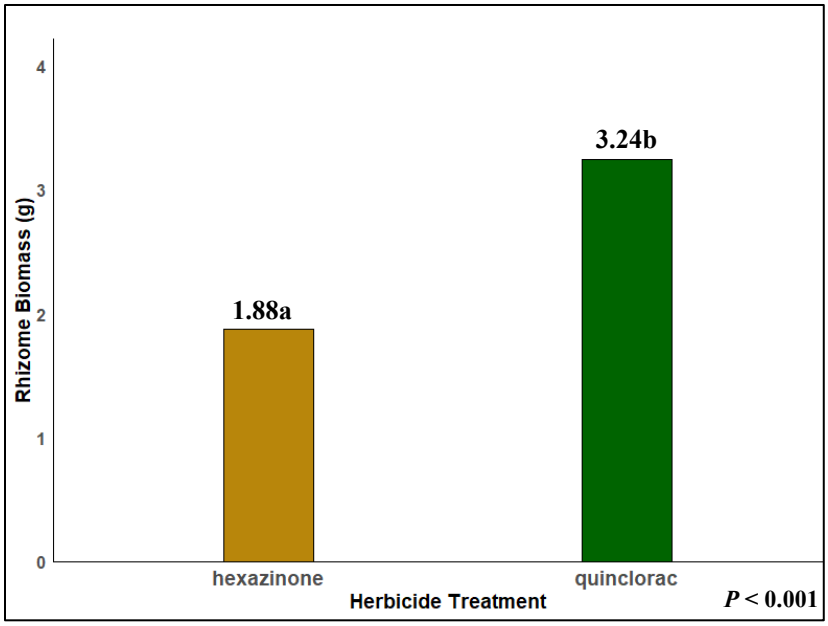

Figure 3-5: Knotroot foxtail rhizome biomass (g) response to herbicide 51 DAT in 2023. *Note: control treatment was not included in the 2023 preliminary run.*

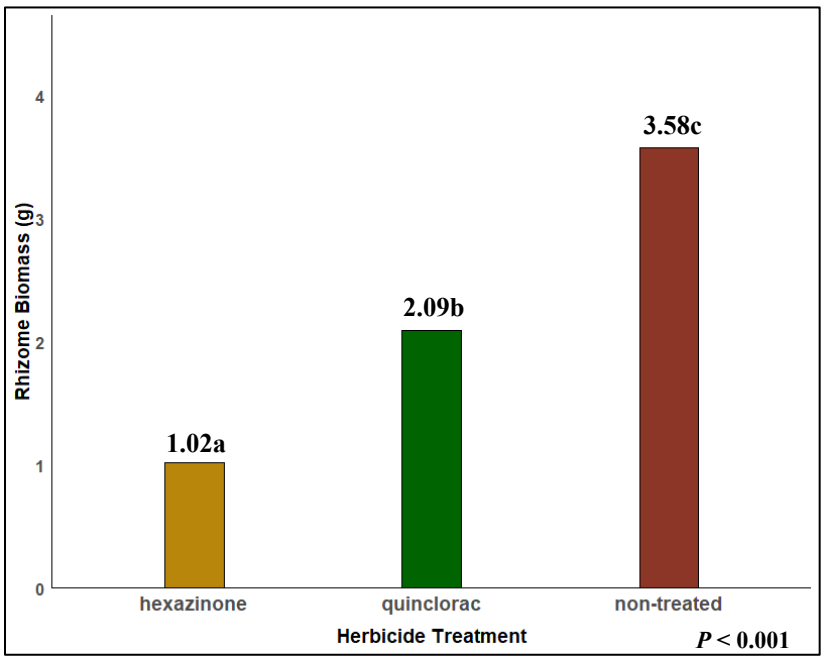

Figure 3-6: Knotroot foxtail rhizome biomass (g) response to herbicide at 51 DAT in 2024

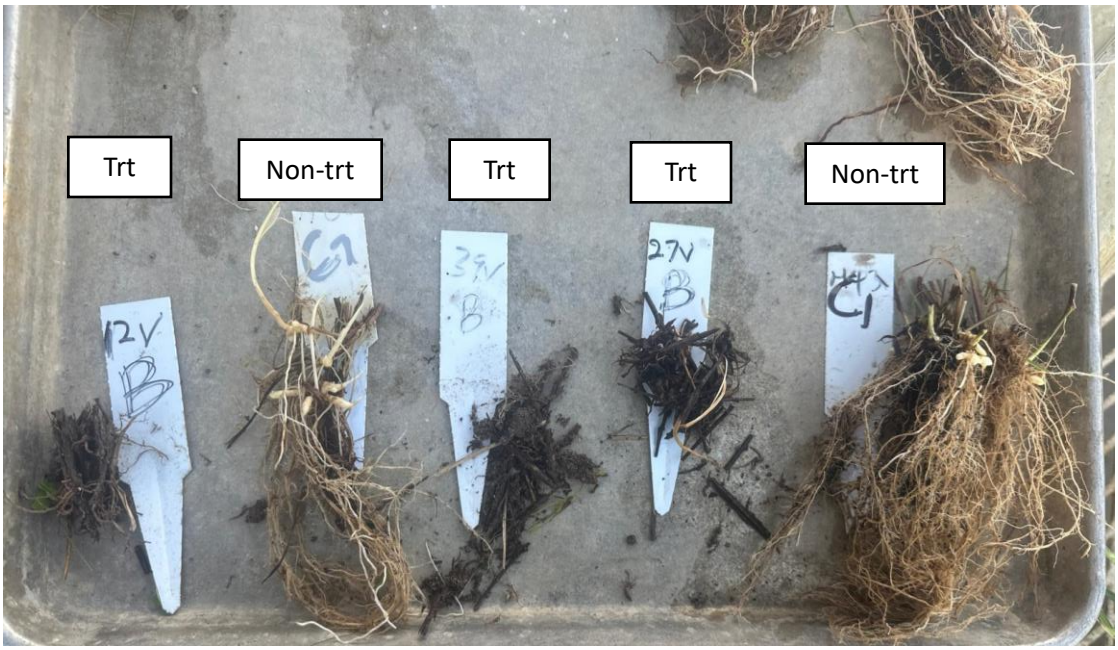

Figure 3-7: Knotroot foxtail fresh rhizome biomass of treated and non-treated plant at 51 days after herbicide treatment in 2024. Trt = rhizome biomass hexazinone treated knotroot foxtail plants, Non-trt = rhizome biomass from non-treated knotroot foxtail plants

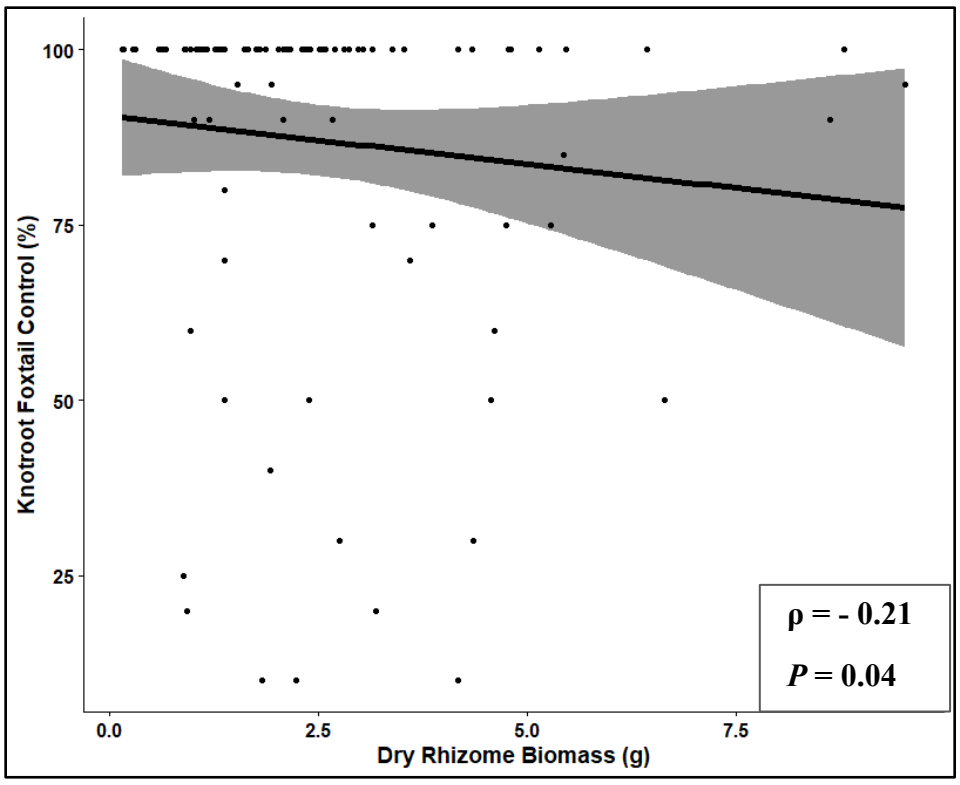

Figure 3-8: Correlation plot showing the relationship between Knotroot foxtail control and dry rhizome biomass (g) at 51 days after herbicide treatment in 2023; rho (ρ) = Spearman's rank correlation coefficient; *P* = p-value at 0.05 level of significance.

(*Note*: ρ ranges from -1 to 1 where ρ = -1 means a perfect negative correlation, 0 = no correlation, and 1 = perfect positive correlation among the two variables). As dry rhizome biomass increased, knotroot foxtail control slightly decreased. The relationship was weak but statistically significant.

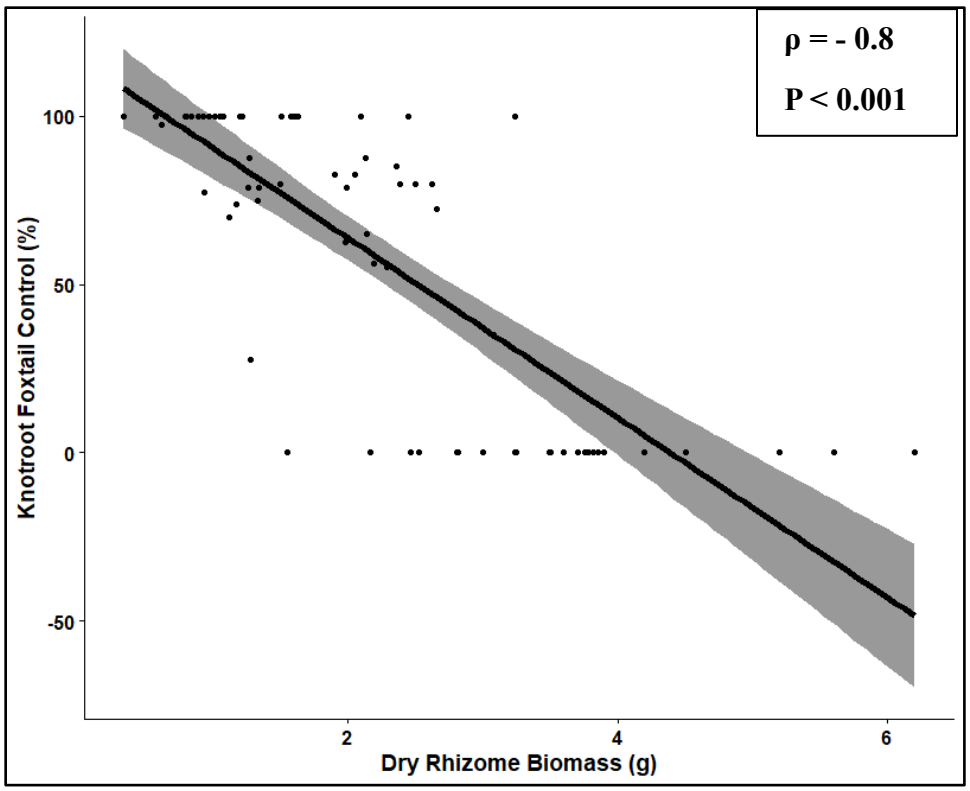

Figure 3-9: Correlation plot showing the relationship between Knotroot foxtail control and dry rhizome biomass (g) 51 days after herbicide treatment in 2024; rho (ρ) = Spearman's rank correlation coefficient; *P* = p-value at 0.05 level of significance.

Note: ρ ranges from –1 to 1, where ρ = –1 means a perfect negative correlation, 0 = no correlation, and 1 = perfect positive correlation between two variables. As dry rhizome biomass increased, knotroot foxtail control strongly decreased. The relationship was strong and statistically significant.