# Influence of Aminocyclopyrachlor plus Metsulfuron on Seedhead Development and Forage Quality in Tall Fescue

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## ABSTRACT

This research quantified the level of seedhead suppression, injury, total ergot alkaloid changes, and forage quality changes in endophyte-infected tall fescue resulting from spring applications of aminocyclopyrachlor (ACP) plus metsulfuron (MET). Results indicated that ACP plus MET reduced tall fescue seedhead density 53 to 55% but also reduced yield 39 to 51% at spring harvest. ACP plus MET also reduced total ergot alkaloid concentration 28 to 34% and improved crude protein (CP) and relative forage quality (RFQ).

This research also determined the impact of ACP plus MET application timing and rate on tall fescue injury and weed control. Spring and early fall applications generally resulted in the greatest tall fescue discoloration and stunting while late fall applications resulted in the least discoloration and stunting. Increasing rates of ACP plus MET generally resulted in greater tall fescue injury in the spring, summer, and early fall application timings. ACP plus MET applied in the summer controlled horsenettle.

The third portion of this research determined the influence of fall vs. spring application timings of ACP plus MET on tall fescue seedhead suppression, injury, forage yield, and forage quality. Results indicated that ACP plus MET applied in late fall and early spring reduced tall fescue seedheads, but the late fall application had less of an impact on spring harvest yield. Increasing rates of ACP plus MET resulted in greater seedhead reduction and yield reduction. ACP plus MET improved CP and RFQ to a greater extent when applied in the spring than in the fall. Increasing ACP plus MET rate also led to higher CP and RFQ.

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# Part 1

# Introduction

## Tall Fescue

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is one of the most predominant cool-season perennial grasses, with 35 million acres grown in the United States (Buckner et al. 1979). It is native to Europe and was being tested in several states by the late 1800s (Buckner et al. 1979). An ecotype was found growing on a Kentucky pasture in 1931 that stayed green all winter. After several years of testing, the cultivar “Kentucky 31” was released in 1943 (Fergus 1952; Fergus and Buckner 1972). During the 1940s and 1950s, the grass became prevalent throughout the southeastern United States, due to its dependability, adaptability to a wide range of soils, and ability to provide grazing mass over much of the year (Hoveland 2010). Tall fescue pastures support approximately 8.5 million beef cows and 700,000 horses in the United States (Hoveland 1993). The grass is now found on approximately 3.5 million acres in Tennessee. In addition to the popularity of tall fescue in pastures, it is also used for turf and roadside cover.

Although tall fescue occurs throughout the country, the principal area of adaptation is in the humid transition zone of the east-central and mid-southern United States, commonly referred to as the “fescue belt” (Hannaway et al. 2010; West 1998; West and Waller 2007). The main factors limiting tall fescue adaptation and suitability are temperature extremes, soil water availability, and soil physical and chemical properties (Hannaway et al. 2010). Optimum growth of tall fescue occurs at ambient temperatures of 20-25 C, but leaves can grow in the range of 4-35 C (Volenec et al. 1984). Tall fescue typically requires at least 375-450 mm of annual precipitation, but up to 900 mm may be needed in areas of high evapotranspiration (Hannaway et al. 1999). Tall fescue is relatively tolerant of drought due to its deep and extensive root system. It can tolerate soil pH range of 4.7 to 9.5 and drainage classes ranging from poorly drained to excessively drained (Hannaway et al. 1999).

## Fescue Endophyte

Despite chemical measures of forage quality that indicate tall fescue forage has potential for high quality and should give good animal performance, it has gained a reputation for poor gains and reproduction problems in cattle (Ball et al. 2007). In 1973, scientists from the USDA Russell Research Center in Athens, GA confirmed that 100% of plants in pastures with cattle showing toxicosis symptoms were infected with a fungal endophyte, while pastures with healthy cattle had a much lower infestation rate (Bacon et al. 1977). The fungus was initially named *Epichloë typhina* (Pers., Fr.) Tul., then renamed *Acremonium coenophialum* Morgan-Jones and Gams, then *Neotyphodium coenophialum* [(Morgan-Jones and Gams) Glenn, Bacon, and Hanlin comb. nov.], and finally *Epichloë coenophiala* (Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov. Endophyte-infected (E+) tall fescue is associated with three cattle disorders: fescue foot, fat necrosis, and fescue toxicosis. Fescue foot is characterized by vasoconstriction at the extremities and gangrene that can result the sloughing of hooves (Bush et al. 1979). Fat necrosis occurs when hardened fat masses form in adipose tissue surrounding the intestine, causing calving and digestive problems (Bush et al. 1979). Fescue toxicosis is more widespread than the other disorders and is detrimental in numerous ways. Steers grazing on heavily infested tall fescue pastures have significantly lower average daily gain (ADG) and exhibit typical toxicosis symptoms: elevated body temperature, excessive salivation, nervousness, and rough hair coats that do not shed in spring (Ball et al. 2007; Hoveland et al. 1980, 1983). Schmidt et al. (1986) reported that pregnancy rates of cows grazing non-infected (E-) tall fescue were 96%

while those grazing E+ tall fescue were 55%. Fescue toxicosis is most noticeable during warmer months and is also known as summer slump. In the US, reduced beef cow conception rates and calf weaning weights caused by fescue toxicity result in losses of $900 million annually (Ball et al. 2007).

The origin of the endophyte has been traced to Europe and it is disseminated only by seed (Siegel et al. 1984). The high infestation rate of US pastures (60-80%) is probably due to the high demand for seed in the mid-1900s, where fresh seed was harvested from one highly infested site in Kentucky and planted the same year (Ball et al. 2007; Siegel et al. 1984). Endophyte hyphae are found only in intercellular spaces of plant tissue and are not visible on the outside of the plant. Thus, presence of the endophyte can only be determined by laboratory examination (Ball et al. 2007; Barker et al. 2010). In seed, the mycelium extends to the apical meristem of the seedlings. In mature plants, hyphae spread by intercalary extension at the same rate as growing leaves (Barker et al. 2010; Christensen and Voisey 2010; Schmid and Christensen 1999). The only naturally occurring route of dissemination of the endophyte is vertical transmission, where fungal hyphae in inflorescences colonize host embryos as they are developing (Christensen and Voisey 2010). The concentration of the endophyte is highest in leaf sheaths and seed (Siegel et al. 1984).

The endophyte produces ergot alkaloids that are detrimental to livestock. Ergot alkaloids are synthesized from L-tryptophan and dimethylallyl diphosphate derived from 3R-mevalonic acid (Schiff 2006). They contain a tetracyclic ergoline ring system (or precursor) and include clavines, lysergic acid and dervatives, and ergopeptine alkaloids (Bush and Fannin 2010; Schiff 2006). Ergopeptines all have a proline attached to a tricyclic peptide with the other two positions occupied by different amino acids. Studies have indicated that the ergopeptines ergovaline and ergotamine are more potent vasoconstrictors of bovine venous tissues than lysergic acid and may also accumulate in tissues over time (Klotz et al. 2006, 2007, 2008; Solomons et al. 1989). However, other research has reported that lysergic acid and lysergol are transported across ruminal and omasal tissues to a greater extent than ergopeptine alkaloids (Hill et al. 2001). Whether fescue toxicosis is caused primarily by a specific agent or the entire class of ergot alkaloids is still unclear (Hill 2005).

Ergot alkaloid concentrations in order from greatest to least are seedhead and seed, crown, stems, leaves, and roots (Azevedo and Welty 1995; Rottinghaus et al. 1991). Ergot alkaloid concentrations in tall fescue typically vary seasonally according to a bimodal curve. In Georgia, Belesky et al. (1988) reported that ergopeptine alkaloid concentrations in grazed tall fescue increased in late spring to coincide with anthesis, decreased by half in summer, and increased again to a maximum in early autumn. Another study in Missouri nongrazed pastures reported ergovaline concentrations followed the same seasonal pattern, but were ten times higher in the spring than in summer (Rottinghaus et al. 1991). High ergovaline levels during the spring were attributed to reproductive maturity of tall fescue, as seedheads had much higher concentrations in the seedhead than in the leaf blade (Rottinghaus et al. 1991). Rogers et al. (2011) reported that frequent clipping of tall fescue resulted in a spring peak absence of ergovaline concentrations, but had little effect on the bimodal pattern of total alkaloid concentrations. The researchers suggested that ergovaline concentrations were low during the spring because host plants were not allowed to develop seedheads and they were forced to prioritize resources toward leaf growth instead of fungal secondary metabolite production.

Although the endophyte produces alkaloids toxic to livestock, it also confers many benefits to its host for stress tolerance. In the east-central and mid-southern United States, where tall fescue is primarily adapted, several factors can stress the plant. Poor mineral nutrient levels occur in highly-weathered, upland soils while drought and high summer temperatures can exacerbate mid-year declines in forage production. Previous research showed higher yields and longer persistence in E+ stands than E- stands in southern portions of the adaptation zone (Bouton et al. 1993; Joost and Coombs 1988; Read and Camp 1986; West et al. 1993). Enhanced drought tolerance in E+ tall fescue probably involves several factors such as root morphology (Malinowski et al. 1999) and osmotic adjustment (Elmi and West 1995). Endophyte infection also increases mineral uptake (Malinowski et al. 1998, 1999) and tillering (Arachevaleta et al. 1989; West et al. 1993). While the presence of *N. coenophialum* can deter feeding by several insect species (Ball et al. 1994; Breen 1993a, 1993b; Eichenseer et al. 1991; Eichenseer and Dahlman 1992; Johnson et al. 1985; Latch et al. 1985; Pennell and Ball 1999; Popay et al. 2005) and confers resistance to some plant parasitic nematodes (Elmi et al. 2000; Kimmons et al. 1990; West et al. 1988), the number of diseases influenced by the endophyte is small (Latch 2010). Although E+ tall fescue can improve stand vigor, the threat of fescue toxicosis to livestock has encouraged research into limiting alkaloid levels in forage systems.

## Mitigating Harmful Effects

Several strategies have been employed to reduce the harmful effects of the endophyte. In pasture systems, grazing management and mechanical clipping can be used to ensure animals consume mostly vegetative growth where ergot alkaloids are less abundant. Increasing stocking rate in E+ pastures either improved or had no effect on daily gain while increasing stocking rate in E- pastures decreased gain (Bransby et al. 1988). Monthly clipping has been shown to reduce ergovaline concentrations in spring (Rogers et al. 2011). Combining tall fescue with other species, such as clover, can dilute the amount of ergot alkaloids consumed by livestock and improve performance of steers (Chestnut et al. 1991; McMurphy et al. 1990) and cow-calf pairs (Ellis et al. 1983; Hill et al. 1979; Stricker et al. 1979). Feeding hay can reduce the amount of alkaloids ingested by livestock. Since ergot alkaloids degrade in the presence of light, heat, and oxygen (Garner et al. 1993), concentrations within the plant are reduced during hay curing (Roberts et al. 2009). Other practices include avoiding grazing during periods of high alkaloid concentration and supplementing animal diets with nontoxic forages and feeds.

A more recent approach has been to apply herbicides that can suppress tall fescue seedhead production. Early work focused on seedhead suppression to reduce mowing needs in turf and roadsides. Sulfometuron, metsulfuron, and chlorsufuron rates as low as 8.8 g ai $ha^{-1}$ have been shown to reduce seedheads in tall fescue turf (Dickens 1987). With the discovery that ergot alkaloids accumulate in seedheads, researchers began work in pasture settings to prevent seedhead formation. The application of the growth regulator mefluidide on E+ tall fescue extended the peak quality of forage beyond late spring and also led to greater intake by steers than untreated E+ pastures (Turner et al. 1990a, 1990b). Other researchers have also reported improved average daily gain of animals grazing mefluidide treated pastures than untreated (Allen et al. 1986; Allen et al. 1988; Garrett et al. 1986). Reynolds et al. (1993) showed that increasing clethodim rate and adding crop oil reduced seedhead density, but also reduced dry matter yield. In another study, total ergot alkaloid concentration in forage from plots treated with 45 g $ha^{-1}$ clethodim was significantly less than untreated plots (Rogers 2010).

**Metsulfuron**

Metsulfuron (methyl 2-[[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2yl) amino] carbonyl] amino] sulfonyl] benzoate) is an acetolactate synthase (ALS) inhibitor and provides control of several perennial and brush species (Bradley et al. 2004; Derr 1989). It has been shown to reduce seedhead density of tall fescue and perennial ryegrass and is registered for use in pastures. By 6 WAT, 6.3 and 8.4 g $ha^{-1}$ metsulfuron reduced tall fescue seedhead density 38-77% and 47-81%, respectively (Moyer and Kelley 1995). Aiken et al. (2012) reported that reproductive tiller densities in tall fescue pastures sprayed with 86.8 + 13.2 g $ha^{-1}$ aminopyralid plus metsulfuron were less than 10% of densities in untreated pastures. In the same study, ADG averaged 39% greater in steers grazing on treated pastures than untreated. Sather et al. (2013) reported metsulfuron-containing herbicides applied to vegetative tall fescue in the spring reduced seedhead densities 14 to 61%. Goff et al. (2014) reported fall applications of aminopyralid plus metsulfuron at rates of 43 + 7 and 87 + 13 g $ha^{-1}$ reduced tall fescue seedheads 52 and 64%, respectively. In the same study, the same rates of aminopyralid plus metsulfuron applied in spring to vegetative tall fescue reduced seedheads only 33%, which was less than fall treatments.

Metsulfuron has also demonstrated potential to injure tall fescue. In a Missouri study, metsulfuron applied at a rate of 0.01 kg $ha^{-1}$ injured tall fescue 22% one month after May application, but only 1% one month after August application (Payne et al. 2010). The absence of injury following the summer application was attributed to semi-dormancy and tall fescue not as actively growing as during the spring application. Metsulfuron at 60 g ai $ha^{-1}$ injured newly seeded fescue 76-93% three weeks after treatment and resulted in significant yield reductions the following year (Peters et al. 1989). Sather et al. (2013) reported spring applications of metsulfuron-containing herbicides reduced tall fescue height 13 to 40%. Another study reported

50-58% and 58-75% injury to tall fescue three weeks after treatment with 6.3 and 8.4 g $ha^{-1}$ metsulfuron, respectively (Moyer and Kelley 1995). Spring applications of metsulfuron at 4.2-8.4 kg $ha^{-1}$ reduced spring forage yields 31-60%, but most of the treatments containing metsulfuron also increased crude protein concentration (Moyer and Kelley 1995).

## Aminocyclopyrachlor

Aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid) belongs to a new class of synthetic auxins called pyrimidine carboxylic acids (Finkelstein et al. 2008). The chemical structure is similar to the pyridine carboxylic acids; however, it contains an additional nitrogen in the heterocyclic ring and a cyclopropyl side chain. Aminocyclopyrachlor is rapidly absorbed by leaves and roots and is translocated in both xylem and phloem to meristematic regions of the plant. The herbicide is thought to target a family of auxin receptor complexes, where it disrupts the hormonal balance necessary for normal root and shoot development (Finkelstein et al. 2008). Additionally, aminocyclopyrachlor effects on the transcription process may be more intense than other compounds with a similar mode of action (Finkelstein et al. 2008). Favorable aspects of the herbicide include low mammalian toxicity, low use rates, and grass tolerance at low rates (Finkelstein et al. 2008).

Aminocyclopyrachlor is currently registered for vegetation management in non-crop areas. Although not registered in pastures, aminocyclopyrachlor has been shown to control a number of broadleaf and brush species in this setting (Alford et al. 2012; Estes and McCarty 2012; Phillips et al. 2012). Since synthetic auxin herbicides are often applied in combination with metsulfuron in pastures, further research is warranted to investigate the impacts of aminocyclopyrachlor plus metsulfuron applied to tall fescue.

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## Part 2

## Effects of Aminocyclopyrachlor plus Metsulfuron on Tall Fescue Yield, Forage Quality, and Ergot Alkaloid Concentration

## Abstract

Most tall fescue in the United States is infected with a fungal endophyte which imparts certain advantages to the plant such as drought tolerance, insect feeding deterrence, and enhanced mineral uptake. However, the endophyte also produces ergot alkaloids that are harmful to livestock and contribute to fescue toxicosis. Since the alkaloids are concentrated in seed and stems, a potential way to reduce the likelihood of fescue toxicosis is by suppressing seedhead formation with herbicides. Research was conducted from 2012-2014 using metsulfuron applied alone and in combination with other herbicides in spring to determine the growth response of tall fescue, effects on forage quality, and ergot alkaloid concentration. Clipping or metsulfuron applied alone or in combination with aminocyclopyrachlor or aminopyralid reduced seedhead density by 36 to 55% compared to the nontreated control. Treatments containing metsulfuron reduced spring harvest yield 35 to 61%, but no differences were observed in the summer or year-after harvests. The same treatments increased crude protein levels by 1.03 to 2.14% and reduced acid detergent fiber levels by 1.60 to 2.76% compared to the nontreated control at spring harvest. Treatments containing metsulfuron reduced ergot alkaloid concentration 26 to 34% at the spring harvest, but no differences were observed in summer-harvested forage. Results from this study indicate metsulfuron applied alone or in combination with aminocyclopyrachlor or aminopyralid can potentially reduce the severity of fescue toxicosis and improve forage quality.


**Nomenclature:** Aminocyclopyrachlor; aminopyralid; metsulfuron; tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.


**Key words:** Fescue toxicosis, forage improvement, hay fields, nutritive value, pastures, seedhead suppression

## Introduction

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is one of the most predominant cool-season perennial grasses, with 14 million ha grown in the United States (Buckner et al. 1979). Tall fescue pastures support approximately 8.5 million beef cows and 700,000 horses in the United States (Hoveland 1993). During the 1940s and 1950s, the Kentucky 31 (KY31) cultivar became prevalent throughout the southeastern United States, due to its dependability, adaptability to a wide range of soils, and ability to provide grazing mass over much of the year (Stuedemann and Hoveland 1988). The persistence of tall fescue in the humid transition zone is mainly due to the presence of a fungal endophyte [*Epichloë coenophiala* (Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov., formerly *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin]. The symbiotic relationship imparts certain advantages to the plant such as drought tolerance (West et al. 1993), insect feeding deterrence (Funk et al. 1993; Johnson et al. 1985), and enhanced mineral uptake (Malinowski et al. 1998, 1999).

Despite the above characteristics and the fact that well-managed tall fescue has good quality indicators for crude protein, digestible dry matter, and mineral content (Bush and Buckner 1973), the grass has been associated with poor animal performance. Fescue toxicosis is an animal disorder that has common symptoms of intolerance to heat, rough hair coats, nervousness, reduced conception rates, and reduced average daily gain (ADG) (Stuedemann and Hoveland 1988). The malady costs American livestock producers between $600 million and $1 billion annually (Fribourg and Waller 2005; Roberts and Andrae 2010). The same endophyte that confers beneficial characteristics to tall fescue also produces ergot alkaloids that are toxic to livestock.

Several strategies have been employed to reduce the harmful effects of the endophyte. Renovation with endophyte-free or novel-endophyte tall fescue is an option in certain situations. However, endophyte-free tall fescue is less persistent than endophyte-infected tall fescue, especially in more southern regions or on rocky soils (Bransby et al 1988; Read and Camp 1986; Roberts and Andrae 2010). Additionally, endophyte-free tall fescue stands can be readily contaminated with endophyte-infected volunteer tall fescue by seed remaining in the soil seed bank or by seed from nearby plants (Barker et al. 2005). Even replacement with novel-endophyte tall fescue may not be adopted due to complex renovation processes and high seed costs (Roberts and Andrae 2005).

When pasture renovation is not feasible, tall fescue production should include strategies related to alkaloid management, a concept that focuses on minimizing toxicity (Roberts and Andrae 2010). Ergot alkaloids are found with highest concentrations in tall fescue seedheads (Rottinghaus et al. 1991). Accordingly, ergot alkaloid concentrations in tall fescue pastures are high in late spring, when seedheads are present (Belesky et al. 1988; Rottinghaus et al. 1991). Compounding the problem of fescue toxicosis is the fact that tall fescue seedheads are often selectively grazed by cattle and horses (Aiken et al. 1993; Goff et al. 2012). In pasture systems, grazing management and mechanical clipping can be used to ensure animals consume mostly vegetative growth where the alkaloids are less abundant. Another alkaloid management strategy is suppressing seedhead formation with herbicides. Early research reported mefluidide applications suppressed tall fescue seedheads and improved forage quality (Glenn et al. 1980; Reynolds et al. 1993a; Turner et al. 1990). Other studies have shown that clethodim, haloxyfop, and sethoxydim also reduced seedhead density and improved forage quality (Reynolds et al. 1993a, 1993b).

Recent research has focused on seedhead suppression with metsulfuron. The herbicide is an acetolactate synthase (ALS) inhibitor and provides control of several perennial broadleaf and brush species (Bradley et al. 2004; Derr 1989; Ferrell et al. 2009). In one study, 6 and 8 g $ha^{-1}$ metsulfuron reduced tall fescue seedhead density 38 to 77% and 47 to 81%, respectively (Moyer and Kelley 1995). Sather et al. (2013) reported metsulfuron-containing herbicides applied to vegetative tall fescue reduced seedhead density 14 to 61%. Researchers in Kentucky have also reported seedhead suppression with aminopyralid plus metsulfuron (Aiken et al. 2012; Goff et al. 2014). Although metsulfuron reduces tall fescue seedhead density, it also reduces forage yield (Moyer and Kelley 1995; Sather et al. 2013). As indicated by Aiken and Strickland (2013), research is needed to determine whether yield reductions can be attributed to a reduction in the presence of seedheads and stems or a direct negative effect on vegetative growth rates. Research is also needed to determine effects of different herbicides and rates on ergot alkaloids in fresh harvested forage.

Aminocyclopyrachlor, hereafter abbreviated ACP, is a synthetic auxin herbicide that has been registered for use in non-cropland and right-of-way applications (Anonymous 2014). One potential herbicide application to pastures is a combination of ACP plus metsulfuron. Previous research has been conducted to evaluate the efficacy of ACP plus metsulfuron on pasture weeds, but has not included impacts on other aspects of forage production. Therefore, the objective of this study was to determine the effects of metsulfuron applied alone and in combination with ACP or aminopyralid on tall fescue injury, seedhead density, total yield, leaf yield, forage nutritive values, and ergot alkaloid concentration.

## Materials and Methods

Research was initiated in March 2012 and repeated in April 2013 at the East Tennessee Research and Education Center near Alcoa, TN (35.844$^{o}$N, 83.965$^{o}$W) and the Plateau Research and Education Center near Crossville, TN (36.014$^{o}$N, 85.129$^{o}$W). The predominant soil types at Alcoa sites were Decatur silty clay loam and Alcoa loam, while the predominant soil at Crossville was a Lily loam. The sites chosen were well-established KY31 tall fescue pastures with at least 85% endophyte infestation level as determined by microscopic analysis of tillers. Each site was mowed in winter prior to trial initiation to 15 cm stubble height. Individual treated plot size was 1.8 by 6.1 m with running checks 0.5 m wide on both sides of the plot. Plots were arranged in a randomized complete block design with four replications. Nitrogen was applied two weeks after herbicide application at a rate of 67 kg ha$^{-1}$. Livestock were excluded for the duration of the experiment.

Herbicide treatments were ACP (DPX-MAT28 50SG Herbicide, DuPont, Wilmington, DE 19898) plus metsulfuron (Ally XP Herbicide, DuPont, Wilmington, DE 19898) at 47 + 7 and 78 + 12 g ai ha$^{-1}$, metsulfuron alone at 7 and 12 g ai ha$^{-1}$, aminopyralid plus metsulfuron (Chaparral Specialty Herbicide, Dow AgroSciences, Indianapolis, IN 46268) at 77 + 12 g ai ha$^{-1}$, and ACP plus 2,4-D (DPX-RRW97 Herbicide, DuPont, Wilmington, DE 19898) at 78 + 591 g ai ha$^{-1}$. Rates were based on anticipated use rates of ACP plus metsulfuron, where 47 + 7 and 78 + 12 g ha$^{-1}$ correspond to 0.6x and 1x use rates, respectively. ACP plus 2,4-D was included as a comparison herbicide treatment that did not contain metsulfuron. At herbicide application, tall fescue was 15 cm in height and in the vegetative growth stage (Table 1). Early spring herbicide application coincided with recommended timing for control of several weeds, including musk thistle (*Carduus nutans* L.), buckhorn plantain (*Plantago lanceolata* L.), and buttercup species

(*Ranunculus* spp.). Herbicides were applied with a $CO_2$ backpack sprayer and a four-nozzle boom calibrated to deliver 140 L $ha^{-1}$ of spray solution which included non-ionic surfactant at 0.25% v/v. A clipping treatment was included to compare chemical seedhead suppression with mechanical removal. Plots were clipped at the early boot stage of tall fescue, approximately two weeks before harvest using a sicklebar mower. Clipping height was 25 $\pm$ 3 cm, which was ~ 3 cm above the leaf canopy, in order not to interfere with later yield measurements.

Tall fescue discoloration and stunting were visually evaluated at 2, 4, 8, 16, and 52 weeks after treatment (WAT) on a 0-99% scale. Discoloration was based on yellowing, browning, and desiccation of tall fescue leaves, whereas stunting was based on plant height and vigor. Plots were harvested once in the spring and once in the summer to determine seedhead density, yield, forage quality, and total ergot alkaloid concentration. At each harvest time, two 0.09 $m^2$ sample areas in each plot were clipped using hand trimmers at a height of 12 $\pm$ 1 cm. The plant material was separated into tall fescue leaves, tall fescue stems (including seedheads), and other plant matter, then dried and weighed. Leaf blades that were attached to stems were removed at the collar. A 0.9 m wide strip in the center of each plot was harvested using a Carter forage harvester (Carter Mfg. Co., Brookston, IN 47923) and weighed for fresh weight. The remaining study area was then harvested in the same manner and biomass was removed to simulate a typical hay harvest. At approximately 2 months and 6 months after treatment (MAT), tall fescue tiller density was determined by counting tillers present in two 0.09 $m^2$ sample areas in each plot. The following spring, approximately 14 MAT, plots were harvested again to determine yield, forage quality, and tiller density.

Subsamples of harvested forage were placed in paper bags and dried in a forced air oven at 60 C for 72 h and moisture content determined. The dried subsamples were ground using a

Wiley mill (Thomas Scientific, Swedesboro, NJ 08085) to pass a 2 mm screen and then ground using a cyclone mill (UDY Corporation, Fort Collins, CO 80524) to pass a 1 mm screen and stored in sealed bags at room temperature. Ground samples were scanned from 1100 to 2500 nm to determine log reflectance$^{-1}$ using a near-infrared reflectance spectroscopy (NIRS) instrument (FOSS 5000, FOSS NIRSystems, Inc., Laurel, MD 20723) while running WinISI II software (Infrasoft International LLC, State College, PA 16801). Forage quality constituents of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, calcium (Ca), magnesium (Mg), and relative forage quality (RFQ) were estimated using prediction equations for mixed hay (2013) developed by the NIRS Forage and Feed Testing Consortium (NIRSC, Hillsboro, WI) and are reported on a dry matter (DM) basis. Spectral data were subjected to principal component analysis and all predicted nutritive values for forage samples fit the supplied equations (H-statistics for all samples were < 3.0).

For ergot alkaloid determination, additional subsamples of fresh harvested forage were sealed in plastic bags and immediately stored in a dry ice cooler to prevent alkaloid degradation. Plastic bags were then frozen and stored at -21 C until processing. Samples were freeze-dried at -30 C for 14 d and then ground to 1 mm particle width in the manner as previously described. Total ergot alkaloid concentrations were determined according to the procedure described by Hill and Agee (1994) using a commercial ELISA test kit (Agrinostics Ltd., Watkinsville, GA 30677).

All data were subjected to ANOVA using PROC GLIMMIX (SAS Version 9.4, SAS Institute, Inc., Cary, NC 27513). Each site-year combination was considered an environment sampled at random, as suggested by Carmer et al. (1989) and Blouin et al. (2011). Treatment was considered a fixed effect, while environment and replication (nested within environment) were considered random effects. Tall fescue injury data were arcsin square-root transformed prior to analysis to improve normality, though nontransformed means are presented for clarity. Means from all data were separated using Fisher's Protected LSD test at $P \leq 0.05$.

## Results and Discussion

### Tall Fescue Injury

Visual injury to tall fescue was observed with all treatments containing metsulfuron through the summer harvest (Table 2). At 2 WAT, treatments containing 12 g $ha^{-1}$ metsulfuron resulted in higher discoloration and stunting compared to treatments containing 7 g $ha^{-1}$ metsulfuron. By 4 WAT, stunting was 49 to 53% with treatments containing 12 g $ha^{-1}$ metsulfuron. By 8 WAT, the highest stunting observed was 28%, indicating tall fescue had partially recovered. Moyer and Kelley (1995) reported 58 to 75% tall fescue injury at 3 WAT with 8 g $ha^{-1}$ metsulfuron, while Payne et al. (2010) observed 22% injury at 1 month after treatment with 10 g $ha^{-1}$ metsulfuron. Our results fall within the range of injury reported in these previous studies. At the summer harvest, tall fescue was nearly fully recovered, as maximum discoloration and stunting was 6 and 8%, respectively. Payne et al. (2010) also found that tall fescue had nearly recovered at 5 MAT, where injury was < 5% with 10 g $ha^{-1}$ metsulfuron applied in the spring. At one year after treatment (YAT), no discoloration or stunting was observed (data not shown). These results corroborate with those of Sather et al. (2013), who found no tall fescue height differences with the nontreated control at 1 YAT after spring applications of several metsulfuron-containing herbicides.

### Seedhead Density

Clipping and metsulfuron applied alone or in combination with ACP or aminopyralid reduced tall fescue seedheads 36 to 55% compared to the nontreated control (Table 3). Although visual injury evaluations were different for treatments containing metsulfuron at 7 g $ha^{-1}$ compared to 12 g $ha^{-1}$, seedhead reduction was similar among these treatments, indicating treatments containing 7 g $ha^{-1}$ metsulfuron may adequately reduce seedheads with less injury to tall fescue. Moyer and Kelley (1995) observed 47 to 81% seedhead reduction with 8 g $ha^{-1}$ metsulfuron. Sather et al. (2013) reported 40 to 61% seedhead reduction with rates of metsulfuron ranging from 13 to 21 g $ha^{-1}$ applied alone or in combination with aminopyralid at spring vegetative stage. In the same study, no differences were found within those treatments, also indicating that seedhead suppression can be achieved with lower rates of metsulfuron. Results from Kentucky studies have also shown aminopyralid plus metsulfuron reduces tall fescue seedheads (Aiken et al. 2012, Goff et al. 2014). Treatment with ACP plus 2,4-D resulted in higher seedhead density than the nontreated control. One reason for this observation may be due to reduced broadleaf weed competition in those plots, since development of grass tillers is hindered by shading (Smith and Whitelam 1997).

**Tall Fescue Yield**

Metsulfuron applied alone or in combination with ACP or aminopyralid reduced total biomass yield at spring harvest (Table 3). Yield reduction with treatments containing 7 g $ha^{-1}$ metsulfuron was 35 to 39%, while reduction with treatments containing 12 g $ha^{-1}$ metsulfuron was 46 to 51% from the nontreated control. In a previous study, metsulfuron applied alone at 4 to 8 g $ha^{-1}$ reduced yield 31 to 60% (Moyer and Kelley 1995). Metsulfuron rates ranging from 13 to 21 g $ha^{-1}$ applied alone or in combination with aminopyralid reduced tall fescue yield 43 to 63% (Sather et al. 2013).

Herbicide treatments containing metsulfuron reduced tall fescue stem yield 42 to 67%, but also reduced tall fescue leaf yield 34 to 50% from nontreated. The greatest reduction in yield of other species was 28%, following application of 77 + 12 g $ha^{-1}$ aminopyralid plus metsulfuron. The majority of other plant species present was Kentucky bluegrass (*Poa pratensis* L.) and orchardgrass (*Dactylis glomerata* L.). As tall fescue was injured with treatments containing metsulfuron, the proportion of other species was greater, most likely due to the greater tolerance of Kentucky bluegrass and orchardgrass to metsulfuron (Dernoeden 1990, Witt 2009). At 2 MAT, ACP plus metsulfuron at 47 + 7 g $ha^{-1}$ reduced tall fescue tiller density 20% compared to the nontreated control. As indicated by the reductions in tall fescue leaf yield and tiller density, total biomass yield reduction at spring harvest can be attributed not to just seedhead suppression, but also reductions in tall fescue vegetative growth per unit area. Goff et al. (2014) observed reductions in tall fescue crowns and increased presence of orchardgrass and Kentucky bluegrass in response to aminopyralid plus metsulfuron applied in early spring.

Total biomass yields and tall fescue leaf yields in all treatments were the same at the summer harvest (Table 4). Similarity between yields provides further evidence that tall fescue receiving metsulfuron-containing treatments had recovered by the summer harvest. Additionally, tiller densities in all treatments were the same at 6 MAT. At the year-after harvest, yields and tiller densities in all treatments were the same, as well (Table 5). Sather et al. (2013) also reported no differences in fall and year-after tall fescue yields among metsulfuron-containing herbicides applied in the spring.

**Forage Quality**

At spring harvest, treatments containing metsulfuron resulted in CP content ranging from 14.23 to 15.34%, while CP content in nontreated forage was 13.20% (Table 6). Treatments containing metsulfuron reduced ADF and NDF of harvested forage. ACP plus metsulfuron at 78 + 12 g $ha^{-1}$ and metsulfuron alone at 7 or 12 g $ha^{-1}$ reduced lignin content compared to the nontreated control and ACP plus 2,4-D. NDF estimates the cell wall fraction, or total fiber content, and is used to predict forage intake by animals. ADF estimates the highly indigestible fraction of fiber and is used to predict forage digestibility. As grasses mature, cell walls become lignified and more indigestible than younger, vegetative forage. Additionally, stems have higher fiber and less CP than leaves. Treatments containing metsulfuron resulted in lower stem yields than nontreated forage; therefore, these treatments had greater CP and less fiber than nontreated forage. Moyer and Kelley (1995) found 8 g $ha^{-1}$ metsulfuron increased CP content by as much as 34 g $kg^{-1}$ over the nontreated control. Sather et al. (2013) found that metsulfuron at 13 g $ha^{-1}$ applied alone or in combination with aminopyralid had CP contents of 8.2 and 9.1%, respectively, and were greater than the 6.5% CP found in nontreated forage. These studies also reported similar reductions in fiber content with metsulfuron-containing treatments.

Relative forage quality (RFQ) is an index of forage quality that takes into account CP, intake, and digestibility and predicts animal performance more accurately than relative feed value (Ball et al. 2001). ACP plus metsulfuron at 78 + 12 g $ha^{-1}$ and metsulfuron alone at 12 g $ha^{-1}$ improved RFQ compared to the nontreated control at spring harvest. All metsulfuron-containing treatments improved RFQ compared to ACP plus 2,4-D and clipping treatments. ACP plus metsulfuron at 78 + 12 g $ha^{-1}$ and metsulfuron alone at 7 or 12 g $ha^{-1}$ resulted in greater Ca and Mg content in compared to nontreated.

At summer harvest, CP, ADF, NDF, and RFQ were similar for all treatments (Table 7). By this time, tall fescue had recovered and leaf proportions were similar. Also, stem reductions at this time would not factor into forage quality improvements as with the spring harvest. At year after harvest, CP, ADF, NDF, and RFQ were similar for all treatments ($P > 0.32$, data not shown). Our results indicate that improvements to tall fescue forage quality after applications of metsulfuron-containing treatments are most apparent in the spring harvest.

**Total Ergot Alkaloids**

At spring harvest, treatments containing metsulfuron reduced ergot alkaloid concentration 26 to 34% from the nontreated control (Table 3), probably due to the reduction in seedheads, which have the greatest amount of ergot alkaloids compared to other tall fescue plant parts (Rottinghaus et al 1991). Clipping did not change ergot alkaloids compared to nontreated. Although clipping reduced seedhead density, the clipped seedheads were still present in harvested forage and alkaloids may have only partially degraded by the time of the spring harvest. Additionally, the stems that remained below the clipping height could have contributed to greater alkaloid amounts. Aiken et al. (2012) found that stems had greater ergopeptine concentrations than leaf blades and sheaths. At summer harvest, ergot alkaloid concentrations were the same in all treatments (Table 4). This is likely due to tall fescue being in the vegetative stage of growth and little to no stems or seedheads present in harvested forage.

Metsulfuron applied alone or in combination with ACP or aminopyralid reduced seedhead density and total ergot alkaloid concentrations in spring-harvested tall fescue forage. Forage quality was also greater in all treatments containing metsulfuron due to the low proportion of stems. Animal performance may be improved by consumption of less toxic and more nutritive forage. However, treatments containing metsulfuron also injured tall fescue and reduced yield in the spring. Cattle stocking rate may need to be reduced in the spring due to limited forage availability with these treatments. By late summer, tall fescue had recovered, and forage yields and nutritive values were similar. Metsulfuron applied alone or in combination with

ACP or aminopyralid may be utilized to reduce the severity of fescue toxicosis with no long-term effects on grass productivity.

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# Part 3

## Rate and Application Timing Effects of Aminocyclopyrachlor plus Metsulfuron on Injury and Forage Quality in Tall Fescue

## Abstract

Field trials were conducted in 2012 and repeated in 2013 to determine the impacts of aminocyclopyrachlor plus metsulfuron on tall fescue growth, weed control, and year-after yield and forage quality. Rates of 47 + 7, 78 + 12, and 155 + 23 g ai $ha^{-1}$ aminocyclopyrachlor plus metsulfuron were applied in early spring, summer, early fall, and late fall to tall fescue pastures. Spring applications resulted in rapid development of injury symptoms and stunting reached 60% with the highest herbicide rate at 4 weeks after treatment (WAT). Late fall applications resulted in the lowest visual injury and stunting averaged 13% across all rates at the following spring harvest. Horsenettle control was 95% or greater at 8 WAT with summer applications of aminocyclopyrachlor plus metsulfuron. Aminocyclopyrachlor plus metsulfuron at 78 + 12 and 155 + 23 g $ha^{-1}$ applied in either early fall or late fall reduced tall fescue yields 10 to 25% the following spring. No differences were observed in tall fescue tiller densities at the year-after spring harvest. Compared to the nontreated control, early and late fall applications increased crude protein content by 1.24 and 1.67%, respectively. Results from this experiment indicated tall fescue was able to recover from aminocyclopyrachlor plus metsulfuron applications, although stunting ranged from 13 to 38% at 8 WAT in plots treated in the spring, summer, or early fall. In addition to weed control, aminocyclopyrachlor plus metsulfuron can improve forage quality of tall fescue, but higher rates applied in early or late fall may reduce forage yield the following spring.

**Nomenclature:** Aminocyclopyrachlor; metsulfuron; horsenettle, *Solanum carolinense* L.; tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.

**Key words:** Forage improvement, hay fields, nutritive value, pasture productivity

## Introduction

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is one of the most important grass species utilized in pastoral agriculture in the United States, occupying over 14 million ha and providing a forage base for approximately 8.5 million beef cows and 700,000 horses (Buckner et al. 1979; Hoveland 1993). The Kentucky 31 (KY31) cultivar is widely popular in the mid-South due to its ease of establishment, adaptability to a wide range of soils and climate, and long grazing season (Stuedemann and Hoveland 1988). Much of the success of tall fescue in the humid transition zone is due to the symbiotic relationship with a fungal endophyte [*Epichloë coenophiala* (Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov., formerly *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin]. The presence of the endophyte confers several benefits to the plant, including drought tolerance (West et al. 1993), insect feeding deterrence (Funk et al. 1993; Johnson et al. 1985), and enhanced mineral uptake (Malinowski et al. 1998, 1999).

Tall fescue has been associated with poor animal performance, despite its dependability and high nutritive values for crude protein, digestible dry matter, and mineral content (Bush and Buckner 1973). Fescue toxicosis is an animal disorder that has common symptoms of intolerance to heat, rough hair coats, nervousness, reduced conception rates, and reduced average daily gain (ADG) (Stuedemann and Hoveland 1988). It occurs after livestock consume forage containing toxic ergot alkaloids that are produced by the endophyte (Hill 2005).

One modern strategy to alleviate the severity of fescue toxicosis is the concept of alkaloid management, which focuses on minimizing toxicity in production systems (Roberts and Andrae 2010). One promising component of alkaloid management is chemical seedhead suppression, since tall fescue seedheads contain the highest concentrations of ergot alkaloids (Rottinghaus et al. 1991). Early studies have reported tall fescue seedhead suppression with clethodim, mefluidide, and sethoxydim (Glenn et al. 1980; Reynolds et al. 1993a, 1993b; Turner et al. 1990). However, these active ingredients can substantially reduce tall fescue yield (Reynolds et al. 1993a, 1993b) and provide little to no control of broadleaf weeds (Anonymous 2010a, 2010b, 2013).

Recent tall fescue seedhead suppression studies have focused on herbicides containing metsulfuron (Aiken et al. 2012; Goff et al. 2014; Moyer and Kelley 1995; Sather et al. 2013). Metsulfuron is an acetolactate synthase (ALS) inhibitor and provides control of several perennial broadleaf and brush species (Bradley et al. 2004; Derr 1989; Ferrell et al. 2009). Herbicides containing metsulfuron have been shown to improve tall fescue forage quality, but can also injure tall fescue and reduce yield. The majority of chemical weed control options utilized in cool-season grass pastures and hay fields are synthetic auxin herbicides. However, metsulfuron-containing treatments are often more effective on certain weeds such as nodding spurge [*Chamaesyce nutans* (Lag.) Small] (Rhodes and Phillips 2012) and blackberry (*Rubus* spp.) (Ferrell et al. 2009).

Aminocyclopyrachlor, hereafter abbreviated ACP, is a synthetic auxin herbicide that has been registered for use in non-cropland and right-of-way applications (Anonymous 2014). One potential herbicide application to pastures is a combination of ACP plus metsulfuron. Previous research has shown that ACP plus metsulfuron can be used in different application windows to control weeds with various growth cycles, including blackberries, buttercup species (*Ranunculus* spp.), Canada goldenrod (*Solidago canadensis* L.), common ragweed (*Ambrosia artemisiifolia* L.), dogfennel [*Eupatorium capillifolium* (Lam.) Small], spiny amaranth (*Amaranthus spinosus* L.), and tall ironweed [*Vernonia gigantea* (Walt.) Trel.] (Israel et al. 2012; Rhodes et al. 2013).

However, research is needed to determine the crop response when ACP plus metsulfuron is applied at different times of the year. Additionally, limited research is available on the effects of metsulfuron-containing herbicides on tall fescue injury and yield the following year (Goff et al 2014; Sather et al. 2013). The objective of this study was to determine the effects of ACP plus metsulfuron rates and application timings on tall fescue injury, weed control, and year-after yield and forage quality.

## Materials and Methods

Field trials were conducted in 2012 and repeated in 2013 at the East Tennessee Research and Education Center, near Alcoa, TN (35.844$^{o}$N, 83.965$^{o}$W) and the Plateau Research and Education Center near Crossville, TN (36.014$^{o}$N, 85.129$^{o}$W). The four distinct sites were well-established KY31 tall fescue pastures with at least 85% endophyte infestation level as determined by microscopic analysis of tillers. The predominant soil types at Alcoa sites were Decatur silty clay loam and Alcoa loam, while the predominant soil at Crossville was a Lily loam. Each site was mowed in winter prior to trial initiation to 15 cm stubble height. At the start of the growing season, each site received 67 kg N ha$^{-1}$. Treated plot size was 1.8 m by 6.1 m with running checks of 0.9 m width between each plot. Livestock were excluded for the duration of the experiment.

The experimental design was a randomized complete block with four replications. Treatments were arranged in an augmented two-factor factorial which included a nontreated control for comparison. Factor A consisted of four levels of application timings: early spring, summer, early fall, and late fall (Table 8). The early spring and late fall timings represent recommended application windows in Tennessee for common winter weeds such as buttercup species and musk thistle (*Carduus nutans* L.). The summer and early fall application timings are recommended for troublesome summer perennial weeds such as horsenettle (*Solanum carolinense* L.), tall ironweed, and blackberries (Rhodes and Israel 2014; Rhodes and Phillips 2012). In late spring of each year, all plots were harvested and biomass was removed to simulate a typical hay harvest. Tall fescue height at the time of application ranged from 15 to 23 cm. Factor B consisted of ACP (DPX-MAT28 50SG Herbicide, DuPont, Wilmington, DE 19898) plus metsulfuron (Ally XP Herbicide, DuPont, Wilmington, DE 19898) applied at three rates: 47 + 7, 78 + 12, and 155 + 23 g ai ha$^{-1}$. Herbicide treatments were applied with a $CO_2$ backpack sprayer equipped with four flat-fan nozzles (Teejet XR8002, Spraying Systems Co., Wheaton, IL 60189). Application volume was 140 L ha$^{-1}$ and all herbicide treatments included non-ionic surfactant at 0.25% v/v.

Fescue discoloration and stunting and weed control were evaluated at 2, 4, and 8 weeks after treatment (WAT) on a 0-99% scale. Discoloration was based on yellowing, browning, and desiccation of fescue leaves, whereas stunting was based on plant height and vigor. A weed control evaluation of 0% was equivalent to the weed density and vigor in the nontreated control and 99% was equivalent to no living weeds present in the plot. At six and fourteen months after initial treatment (MAIT), tall fescue tiller density was determined by counting tillers present in two 0.09 m$^2$ sample areas in each plot.

Plots were harvested in the spring of the following year (14 MAIT) to determine yield and forage quality. A 0.9 m wide strip in the center of each plot was harvested using a Carter forage harvester (Carter Mfg. Co., Brookston, IN 47923) and weighed for fresh weight. Hand grab samples of harvested forage were then placed in paper bags and dried in a forced air oven for 72 h at 60 C to determine moisture content. The dried subsamples were ground through a

Wiley mill (Thomas Scientific, Swedesboro, NJ 08085) to pass a 2 mm screen and then ground through a cyclone mill (UDY Corporation, Fort Collins, CO 80524) to pass a 1 mm screen and stored in sealed bags at room temperature. Ground samples were scanned from 1100 to 2500 nm to determine log reflectance$^{-1}$ using a near-infrared reflectance spectroscopy (NIRS) instrument (FOSS 5000, FOSS NIRSystems, Inc., Laurel, MD 20723) while running WinISI II software (Infrasoft International LLC, State College, PA 16801). Forage quality constituents of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, and relative forage quality (RFQ) were estimated using prediction equations for mixed hay (2013) developed by the NIRS Forage and Feed Testing Consortium (NIRSC, Hillsboro, WI) and are reported on a dry matter (DM) basis. Spectral data were subjected to principle component analysis and all predicted nutritive values for forage samples fit the supplied equations (H-statistics for all samples were < 3.0).

All data were subjected to ANOVA using PROC GLIMMIX (SAS Version 9.4, SAS Institute, Inc., Cary, NC 27513). Each site-year combination was considered an environment sampled at random, as suggested by Carmer et al. (1989) and Blouin et al. (2011). Application timing and rate were considered fixed effects, while environment and replication (nested within environment) were considered random effects. Type III statistics were used to test all possible effects of fixed factors and least square means were separated according to a *t* test when timing, rate, or their interaction was significant ($P \leq 0.05$). Weed control evaluations were arcsin square-root transformed prior to analysis to improve normality, though nontransformed means are presented for clarity. Means from weed control data were separated using Fisher's Protected LSD test at $P \leq 0.05$.

## Results and Discussion

### Tall Fescue Injury

A timing by rate interaction occurred when visual injury was evaluated at 2, 4, and 8 WAT. At 2 WAT, the spring application of 155 + 23 g $ha^{-1}$ ACP plus metsulfuron resulted in the highest discoloration and stunting (Table 9). Summer and early fall treatments did not exhibit injury as quickly as spring treatments but resulted in significantly more injury than late fall treatments. Tall fescue grows rapidly in early spring and produces many new leaves. Since sulfonylurea herbicides are translocated to meristems, injury to tall fescue was most noticeable in the spring application, when growth was most rapid.

At 4 WAT, discoloration in spring, summer, and early fall treatments ranged from 16 to 36%. However, discoloration and stunting in late fall treatments were minimal, with only the highest herbicide rate having greater injury than the nontreated control. Stunting was greatest with the spring application of the highest rate. Injury observed in our study was greater than injury reported in a Missouri study, where 10 g $ha^{-1}$ metsulfuron injured tall fescue 22 and 1% at one month after May and August applications, respectively (Payne et al. 2010). However, Moyer and Kelley (1995) reported as much as 75% injury to tall fescue at 3 WAT following a spring application of 8 g $ha^{-1}$ metsulfuron. Stunting of tall fescue was different between herbicide rates within each of the spring, summer, and early fall timings. Other researchers have also observed greater injury to tall fescue with higher metsulfuron rates applied in the spring (Moyer and Kelley 1995; Sather et al. 2013). Within herbicide rates, stunting at 4 WAT was the same for the summer and early fall timings, but was less than stunting for the spring timing.

At 8 WAT, herbicide rates were different in the early fall timing for discoloration, with values ranging from 19 to 36%. In all other timings, discoloration ranged from 8 to 18%.

Stunting was different between herbicide rates within the spring and early fall timings, but differences between herbicide rates for the summer and late fall timings were less apparent. From 4 to 8 WAT, tall fescue appeared to recover at the spring and summer timings. In fact, by November, tall fescue stunting was no more than 10% in plots treated at these timings (data not shown). However, injury was sustained from 4 to 8 WAT with the early fall and late fall timings. Sather et al. (2013) found that August applications of metsulfuron-containing herbicides reduced tall fescue yield in the fall compared to April applications. In a study on perennial ryegrass (*Lolium perenne* L. ssp. *perenne*), James et al. (1999) found that pastures treated with metsulfuron in autumn were slower to recover than pastures treated in spring.

At the following spring harvest, tall fescue injury was not observed in the early spring and summer timings; therefore, these timings were excluded from the analysis. For the early fall and late fall timings, the main effects of timing and herbicide rate were significant for discoloration and stunting ($P \leq 0.01$). Stunting was 4 and 13% for the early and late fall timings, respectively (Table 10). Similarly, stunting ranged from 4 to 13% with the three rates of ACP plus metsulfuron, when averaged across the early and late fall timings (Table 11). Another study reported that April, May, or August applications of several metsulfuron-containing herbicides did not reduce tall fescue height compared to the nontreated control by the following spring (Sather et al 2013).

**Weed Control**

Although the sites for the experiment were chosen based on healthy tall fescue populations, two of the environments had substantial horsenettle populations that were evaluated to determine herbicide efficacy at the summer application timing. At 4 WAT, horsenettle control ranged from 83 to 96% for ACP plus metsulfuron (Table 12). At 8 WAT, horsenettle control was

95% or greater at all rates. Several researchers have reported good control of horsenettle with summer applications of ACP applied alone or in combination with other herbicides (Meredith et al. 2013; Phillips et al. 2012; Warren et al. 2012).

Other weeds were present in one environment and control was determined after spring application. ACP plus metsulfuron controlled Persian speedwell (*Veronica persica* Poir.) 96 to 99% at 4 WAT and 99% at 8 WAT. Buckhorn plantain (*Plantago lanceolata* L.) was controlled 94 to 99% and 99% at 4 and 8 WAT, respectively (data not shown). Other researchers have observed good control of buckhorn plantain with ACP plus metsulfuron in noncropland situations (Belcher and Walker 2011; Wright et al. 2011). At 4 WAT, control of common chickweed [*Stellaria media* (L.) Vill.], hairy buttercup (*Ranunculus sardous* Crantz), and prickly lettuce (*Lactuca serriola* L.) was 97% or greater (data not shown). Excellent control of hairy buttercup with ACP plus metsulfuron has been observed in previous studies (Israel and Rhodes 2015).

**Tall Fescue Tiller Density and Yield**

At 6 MAIT, tiller density was not different between herbicide rates, timings, or their combinations and ranged from 624 to 805 tillers $m^{-2}$ (Table 13). The lack of a response in tiller densities in the fall provides further evidence that tall fescue had recovered from spring- or summer-applied ACP plus metsulfuron. At the spring harvest (14 MAIT), a timing by herbicide rate interaction was observed for yield. ACP plus metsulfuron at 78 + 12 or 155 + 23 g $ha^{-1}$ applied in the early fall or late fall reduced yield compared to the nontreated control. In the late fall timing, yield decreased from 3839 to 3009 kg $ha^{-1}$ as ACP plus metsulfuron rate increased. However, within the other timings, yield was similar for the three rates. Tiller density at 14 MAIT was not different between herbicide rates, timings, or their combinations and ranged from

735 to 954 tillers m$^{-2}$. In a Missouri study, Sather et al (2013) reported tall fescue pastures had recovered in the spring from metsulfuron-containing treatments applied the previous year in April, May, or August. Results from this experiment indicate that ACP plus metsulfuron applied in late September or later can reduce tall fescue yield the following spring.

**Forage Quality**

At the spring harvest the following year, no interaction between timing and rate was observed and the main effect of herbicide rate was not significant for CP, ADF, NDF, lignin, and RFQ ($P \geq 0.21$). However, ACP plus metsulfuron applied in the early or late fall resulted in greater CP and less ADF and NDF than spring and summer treatments (Table 14). NDF is an estimate of the total fiber content of forage, and is used to predict forage intake by animals. ADF is an estimate of highly indigestible cell wall components and is used to predict forage digestibility. Stems are more lignified and have higher fiber and less CP than leafy plant parts. Stunting was still apparent in early and late fall treatments at the time of harvest, indicating tall fescue had not fully recovered. Injury in tall fescue may have resulted in suppressed seedhead formation, which can increase the protein content and reduce the fiber content of harvested forage. Fall applications also resulted in the greatest RFQ, which is calculated from CP, intake, and digestibility and predicts animal performance more accurately than relative feed value (Ball et al. 2001). Previous research has shown that aminopyralid plus metsulfuron applied in the fall reduced tall fescue seedhead density by as much as 60% at the following spring harvest (Goff et al. 2014).

Spring applications of ACP plus metsulfuron resulted in greater CP and lower fiber content than the nontreated control, although differences were small. One possible explanation is that weed populations could have been greater in nontreated plots and accounted for a higher proportion of harvested forage. Our results differ from those of Sather et al. (2013), where there were no differences between spring vegetative, boot stage, and late summer vegetative application timings for CP, ADF, and NDF at year-after harvest the following spring. In that study, later application timings were not included that could have affected stem proportions in the spring.

Results from this study indicate that tall fescue responds differently to ACP plus metsulfuron applied at different times of the year and also different rates. Tall fescue injury was high with spring applications, but recovery was sooner than with fall applications. Fall applications reduced yields the following spring, but also improved forage quality. Tall fescue tiller densities were similar and further suggest long term recovery in tall fescue. Results from this research can help producers predict additional impacts on tall fescue when planning weed control strategies at different times of the year.

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## Part 4

## Impacts of Fall- and Spring-applied Aminocyclopyrachlor plus Metsulfuron on Tall Fescue Seedhead Density, Yield, and Forage Quality

## Abstract

Research was conducted from 2012-2014 with aminocyclopyrachlor plus metsulfuron applied at different rates and timings to determine the effects on tall fescue growth and development and forage quality. Rates of 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g ai ha$^{-1}$ aminocyclopyrachlor plus metsulfuron were applied in late fall and early spring to tall fescue pastures. Late fall treatments did not exhibit as much injury as spring treatments. Stunting at spring harvest ranged from 4 to 42% with all combinations of rate and timing. Timing did not affect seedhead density at harvest as seedheads were reduced 52 and 53% from nontreated for fall and spring applications, respectively. Reductions in spring yield compared to the nontreated control were 19 and 42% for fall and spring applications, respectively. Across both timings, yield reductions ranged from 20 to 39% with aminocyclopyrachlor plus metsulfuron rates of 47+7 to 155+23 g ha$^{-1}$. Relative forage quality (RFQ) of nontreated forage was 105 compared to 114 and 117 for fall and spring applications, respectively. RFQ values ranged from 112 to 120 with aminocyclopyrachlor plus metsulfuron rates of 47+7 to 155+23 g ha$^{-1}$. Aminocyclopyrachlor plus metsulfuron may be applied in the fall to suppress tall fescue seedheads with less injury than spring applications and similar improvements to forage quality.

**Nomenclature:** Aminocyclopyrachlor; metsulfuron; tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.

**Key words:** Fescue toxicosis, forage improvement, hay fields, nutritive value, pastures, seedhead suppression

## Introduction

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] pastures support approximately 8.5 million beef cows and 700,000 horses in the United States (Hoveland 1993). The plant plays a major role in agricultural, turf, and land conservation settings and occupies over 14 million ha (Buckner et al. 1979). In the mid-South, the Kentucky 31 (KY31) cultivar is widespread, because of its persistence, adaptability to a wide range of soils and climate, and long grazing season (Stuedemann and Hoveland 1988). Much of the success of tall fescue in the humid transition zone is due to the symbiotic relationship with a fungal endophyte [*Epichloë coenophiala* (Morgan-Jones & W. Gams) C.W. Bacon & Schardl, comb. nov., formerly *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin]. The presence of the endophyte confers several benefits to the plant such as drought tolerance (West et al. 1993), insect feeding deterrence (Funk et al. 1993; Johnson et al. 1985), and enhanced mineral uptake (Malinowski et al. 1998, 1999). Tall fescue also has good forage quality indicators for crude protein, digestible dry matter, and mineral content (Bush and Buckner 1973).

Despite its dependability and high nutritive values, tall fescue has been associated with poor animal performance. Fescue toxicosis is an animal disorder that has common symptoms of intolerance to heat, rough hair coats, nervousness, reduced conception rates, and reduced average daily gain (ADG) (Stuedemann and Hoveland 1988). The tall fescue endophyte produces ergot alkaloids, which are toxic to livestock (Hill 2005). The cost to American livestock producers is estimated to be from $600 million to $1 billion each year (Fribourg and Waller 2005; Roberts and Andrae 2010).

One option to contend with fescue toxicosis is renovation with endophyte-free or novel-endophyte tall fescue. However, endophyte-free tall fescue is less persistent than endophyte-infected tall fescue, especially in more southern regions or on rocky soils (Bransby et al 1988; Read and Camp 1986; Roberts and Andrae 2010). Additionally, endophyte-free tall fescue stands can be contaminated with endophyte-infected tall fescue from buried seed and tillers or seed dispersed by hay and manure (Barker et al. 2005). Even replacement with novel-endophyte tall fescue may not be adopted due to complex renovation processes and high seed costs (Roberts and Andrae 2005).

Another option to alleviate the severity of fescue toxicosis is the concept of alkaloid management, which focuses on minimizing toxicity in production systems (Roberts and Andrae 2010). One promising component of alkaloid management is chemical seedhead suppression, since tall fescue seedheads contain the highest concentrations of ergot alkaloids (Rottinghaus et al. 1991). Adding to the toxicity risk is the fact that cattle and horses often selectively graze tall fescue seedheads (Aiken et al. 1993, Goff et al. 2012). Early studies have reported tall fescue seedhead suppression with clethodim, mefluidide, and sethoxydim (Glenn et al. 1980; Reynolds et al. 1993a, 1993b; Turner et al. 1990). However, these active ingredients can substantially reduce tall fescue yield (Reynolds et al. 1993a, 1993b) and provide little to no control of broadleaf weeds (Anonymous 2010a, 2010b, 2013).

Recent tall fescue seedhead suppression studies have focused on herbicides containing metsulfuron, an acetolactate synthase (ALS) inhibitor that also controls several perennial broadleaf and brush species (Bradley et al. 2004; Derr 1989; Ferrell et al. 2009). Spring applications of aminopyralid plus metsulfuron reduced reproductive tiller densities by more than 90% compared to nontreated tall fescue (Aiken et al. 2012). Spring applications of metsulfuron-containing herbicides can also increase crude protein content and reduce fiber content of harvested tall fescue forage (Moyer and Kelley 1995, Sather et al. 2013). Unfortunately, yield reductions as high as 60% were reported with spring-applied metsulfuron at 8 g $ha^{-1}$ (Moyer and Kelley 1995). Recently, Goff et al. (2014) observed aminopyralid plus metsulfuron applied in the fall suppressed tall fescue seedheads as much as 60% the following spring, but effects on yield and forage quality were not reported. Further research is warranted to determine the effects of fall-applied metsulfuron on tall fescue seedhead reduction, yield, and forage quality.

Metsulfuron is often applied in combination with auxinic herbicides to obtain broad-spectrum weed control. Aminocyclopyrachlor, hereafter abbreviated ACP, is a synthetic auxin herbicide that has been registered for use in non-cropland and right-of-way applications (Anonymous 2014). One potential application to pastures is a tank mix of ACP plus metsulfuron. Previous research has shown that ACP plus metsulfuron can be applied in the fall or spring to control cool-season weeds such as hairy buttercup (*Ranunculus sardous* Crantz) and bulbous buttercup (*Ranunculus bulbosus* L.) (Israel and Rhodes 2015). However, research is needed to determine other possible benefits to tall fescue forage production. The objective of this study was to determine the effects of ACP plus metsulfuron applied in the fall and spring on tall fescue injury, seedhead density, yield, and forage nutritive values.

## Materials and Methods

Field trials were conducted from 2012-2014 at the East Tennessee Research and Education Center, near Alcoa, TN (35.844$^{o}$N, 83.965$^{o}$W) and the Plateau Research and Education Center near Crossville, TN (36.014$^{o}$N, 85.129$^{o}$W). The predominant soil types at Alcoa were Decatur silty clay loam and Alcoa loam, while the predominant soil at Crossville was a Lily loam. The four distinct sites were well-established KY31 tall fescue pastures with at least 85% endophyte infestation level as determined by microscopic analysis of tillers. Each site was mowed uniformly in mid-fall prior to trial initiation to 15 cm stubble height. At the start of the growing season in the spring, each site received 67 kg N $ha^{-1}$. Treated plot size was 1.8 m by 6.1 m with running checks of 0.9 m width between each plot. Livestock were excluded for the duration of the experiment.

The experimental design was a randomized complete block with four replications. Treatments were arranged in an augmented two-factor factorial which included a nontreated control for comparison. Factor A consisted of two levels of application timings: late fall and early spring (Table 15). These timings represent recommended application windows in Tennessee for common cool-season weeds such as buttercups and musk thistle (*Carduus nutans* L.) (Rhodes and Phillips 2012). Tall fescue height ranged from 13 to 18 cm at the time of application. Factor B consisted of ACP (DPX-MAT28 50SG Herbicide, DuPont, Wilmington, DE 19898) plus metsulfuron (Ally XP Herbicide, DuPont, Wilmington, DE 19898) applied at four rates: 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g $ha^{-1}$. The lower two rates were based on anticipated use rates of ACP plus metsulfuron, corresponding to 0.6x and 1x use rates, respectively. These rates were doubled to simulate instances of sprayer overlap in herbicide application. Herbicide treatments were applied with a $CO_2$ backpack sprayer equipped with four flat-fan nozzles (Teejet XR8002, Spraying Systems Co., Wheaton, IL 60189). Application volume was 140 L $ha^{-1}$ and all herbicide treatments included non-ionic surfactant at 0.25% v/v.

Fescue discoloration and stunting were evaluated at 2, 4, and 8 weeks after treatment (WAT), spring harvest, and summer harvest on a 0-99% scale. Discoloration was based on yellowing, browning, and desiccation of leaves, whereas stunting was based on plant height and vigor. Plots were harvested once in the spring and once in the summer to determine seedhead density, yield, and forage quality. Two 0.09 $m^2$ sample areas in each plot were clipped using hand trimmers at a height of 12 $\pm$ 1 cm, separated, and dried to determine tall fescue seedhead density and yield of fescue leaves, fescue stems (including seedheads), and other plant matter. Leaf blades that were attached to stems were removed at the collar. A 0.9 m wide strip in the center of each plot was harvested using a Carter forage harvester (Carter Mfg. Co., Brookston, IN 47923) and weighed for fresh weight. The remaining study area was then harvested in the same manner and biomass was removed to simulate a typical hay harvest. At two weeks after the spring harvest and one year after the fall application timing, tall fescue tiller density was determined by counting tillers present in two 0.09 $m^2$ sample areas in each plot.

Subsamples of harvested forage were placed in paper bags and dried in a forced air oven for 72 h at 60 C to determine moisture content. The dried subsamples were ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ 08085) to pass a 2 mm screen and then ground through a cyclone mill (UDY Corporation, Fort Collins, CO 80524) to pass a 1 mm screen and stored in sealed bags at room temperature. Ground samples were scanned from 1100 to 2500 nm to determine log reflectance$^{-1}$ using a near-infrared reflectance spectroscopy (NIRS) instrument (FOSS 5000, FOSS NIRSystems, Inc., Laurel, MD 20723) while running WinISI II software (Infrasoft International LLC, State College, PA 16801). Forage quality constituents of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), relative forage quality (RFQ), lignin, calcium (Ca), magnesium (Mg), and phosphorus (P) were estimated using prediction equations for mixed hay (2013) developed by the NIRS Forage and Feed Testing Consortium (NIRSC, Hillsboro, WI) and are reported on a dry matter (DM) basis. Spectral data were subjected to principle component analysis and all predicted nutritive values for forage samples fit the supplied equations (H-statistics for all samples were $< 3.0$).

All data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX (SAS Version 9.4, SAS Institute, Inc., Cary, NC 27513). Each site-year combination was considered an environment sampled at random, as suggested by Carmer et al. (1989) and Blouin et al. (2011). Application timing and rate were considered fixed effects, while environment and replication (nested within environment) were considered random effects. Type III statistics were used to test all possible effects of fixed factors and least square means were separated according to a *t* test when timing, rate, or their interaction was significant ($P \leq 0.05$). An analysis of covariance (ANCOVA) was conducted to determine the effect of timing on seedhead density and total biomass yield with metsulfuron rate as a covariate. Seedhead density and spring harvest total biomass yield means were subjected to linear regression analysis in commercial software (SigmaPlot v. 13, Systat Software, Inc., San Jose, CA 95110).

## Results and Discussion

### Tall Fescue Injury

A timing by rate interaction occurred for all visual injury evaluations except discoloration at 8 WAT. In general, tall fescue treated in late fall did not exhibit injury as quickly as tall fescue treated in early spring. At 2 WAT, discoloration and stunting were mostly the same for all rates of fall-applied ACP plus metsulfuron, but injury increased with herbicide rate for spring treatments (Table 16). In spring, tall fescue was in a stage of rapid vegetative growth and produced many new leaves. Since sulfonylurea herbicides like metsulfuron are translocated to meristems, injury was most apparent in the spring application, when new leaves were rapidly developing.

At 4 WAT, discoloration in fall treatments ranged from 6 to 11%, while discoloration in spring treatments ranged from 12 to 31%. Similarly, the range in stunting was greater in the spring applications compared to fall applications. With spring treatments, tall fescue injury appeared to reach a maximum at 4 WAT. In fact, discoloration at 8 WAT was greater with fall treatments (15%) compared to spring treatments (11%) when averaged across all rates (data not shown). However, stunting at 8 WAT was still greater with spring treatments compared to fall treatments. Moyer and Kelley (1995) observed as much as 75% injury to tall fescue at 3 WAT following a spring application of 8 g ha$^{-1}$ metsulfuron. In a Missouri study, Sather et al. (2013) reported spring applications of metsulfuron-containing herbicides reduced tall fescue height 13 to 40%. Our results confirm that spring applications of metsulfuron can result in considerable injury to tall fescue.

Injury evaluated at spring harvest coincided with 8 WAT for spring applications and approximately 25 WAT for fall applications. Discoloration from fall treatments was minimal, as all rates were the same as the nontreated control. However, stunting was still apparent in tall fescue, even at 25 WAT. In fall treatments, stunting was sustained from 8 WAT to the spring harvest. In spring treatments, tall fescue appeared to recover from 4 WAT to the spring harvest. James et al. (1999) found that perennial ryegrass (*Lolium perenne* L. ssp. *perenne*) pastures treated with metsulfuron in autumn were slower to recover than pastures treated in spring. By the summer harvest, visual injury to tall fescue was minimal, as the highest ACP plus metsulfuron rate applied in the spring resulted in discoloration and stunting evaluations of 3 and 6%, respectively (data not shown). Similar to our results, Payne et al. (2010) reported that tall fescue injury was < 5% at five months after spring application of 10 g ha$^{-1}$ metsulfuron.

## Seedhead Density

Tall fescue seedhead density ranged from 312 for the nontreated to 107 seedheads $m^{-2}$ for 155 + 23 g $ha^{-1}$ ACP plus metsulfuron applied in late fall (Figure 1). Averaged across application timings, seedhead reduction was 33 and 64% with 47 + 7 and 155 + 23 g $ha^{-1}$ ACP plus metsulfuron, respectively. The ANCOVA for tall fescue seedhead density showed no application timing by metsulfuron rate interaction ($P = 0.76$); therefore, only one regression line was fitted. Seedhead density decreased linearly from 285 to 78 seedheads $m^{-2}$ as metsulfuron rate increased from 0 to 23 g $ha^{-1}$. Sather et al. (2013) reported 40 to 61% seedhead reduction with similar rates of metsulfuron (13 to 21 g $ha^{-1}$) applied alone or in combination with aminopyralid at the spring vegetative stage. Moyer and Kelley (1995) reported 47 to 81% tall fescue seedhead reduction with 8 g $ha^{-1}$ metsulfuron applied in the spring. Goff et al. (2014) reported fall applications of aminopyralid plus metsulfuron at rates of 43 + 7 and 87 + 13 g $ha^{-1}$ reduced tall fescue seedheads 52 and 64%, respectively. However, in the same study, the same rates of aminopyralid plus metsulfuron applied in spring to vegetative tall fescue reduced seedheads only ~ 33%, which was significantly less than the fall treatments. Our research indicates that ACP plus metsulfuron applied in the fall or spring to vegetative tall fescue can reduce tall fescue seedhead density.

## Tall Fescue Yield

Total biomass yield at spring harvest ranged from 3412 for the nontreated to 1667 kg $ha^{-1}$ for 155 + 23 g $ha^{-1}$ ACP plus metsulfuron applied in early spring (Figure 2). Yield reduction ranged from 13 to 26% in late fall treatments and 27 to 51% in early spring treatments. The ANCOVA for tall fescue seedhead density indicated a significant interaction between application timing and metsulfuron rate ($P < 0.001$); therefore, regression lines were fitted for both timings. Regression analyses indicated that for every 7 g $ha^{-1}$ increase in metsulfuron rate, 263 kg $ha^{-1}$ of total biomass yield is lost with late fall applications and 539 kg ha$^{-1}$ is lost with spring applications. Moyer and Kelley (1995) reported spring-applied metsulfuron at 4 to 8 g ha$^{-1}$ reduced tall fescue yield 31 to 60%. Sather et al. (2013) reported metsulfuron applied in combination with aminopyralid at metsulfuron rates of 13 to 21 g ha$^{-1}$ reduced tall fescue yield 52 to 63%. Results from our study confirm previous reports that high rates of spring-applied metsulfuron substantially reduce tall fescue yield. However, our study also indicates that late fall applications have less impact than spring applications on tall fescue yield.

At spring harvest, fall-applied ACP plus metsulfuron resulted in tall fescue leaf yield that was the same as nontreated, but spring applications reduced leaf yield 45% from nontreated (Table 17). The main effect of application rate was not significant for tall fescue leaf yield ($P = 0.14$). The main effect of timing was not significant for tall fescue stem yield ($P = 0.35$), but both fall and spring timings averaged < 830 kg ha$^{-1}$, compared to 1945 kg ha$^{-1}$ in the nontreated control. When averaged over application timings, ACP plus metsulfuron at 47 + 7 g ha$^{-1}$ reduced tall fescue stem yield 41%, whereas the three higher ACP plus metsulfuron rates reduced stem yield 62 to 75% from nontreated (Table 18). Yield of other plant species was reduced 26% with spring applications, but the main effect of herbicide rate was not significant ($P = 0.36$). Kentucky bluegrass (*Poa pratensis*) and orchardgrass (*Dactylis glomerata*) were the most predominant species present in other plant matter. Although several broadleaf weed species were controlled by ACP plus metsulfuron (data not shown), few differences were observed in the yield of other species, most likely due to the greater tolerance of Kentucky bluegrass and orchardgrass to metsulfuron (Dernoeden 1990, Witt 2009). Goff et al. (2014) reported increased presence of orchardgrass and Kentucky bluegrass and reductions in tall fescue crowns in response to aminopyralid plus metsulfuron applied in early spring. Averaged over application timings, fewer tall fescue tillers were present in tall fescue treated with ACP plus metsulfuron at 155 + 23 g $ha^{-1}$ compared to the 47 + 7 g $ha^{-1}$ rate. Reduction of total biomass yield may be explained not only by seedhead suppression, but also by reduction of tall fescue vegetative growth per unit area.

At summer harvest, early spring total biomass yields were 21% greater than late fall yields when averaged over application rate (Table 19). The main effect of rate was not significant for total biomass yields ($P = 0.69$), yet when averaged over both application timings, treated tall fescue yields ranged from 1691 to 1825 kg $ha^{-1}$, compared to 1559 kg $ha^{-1}$ in the nontreated control (Table 20). In a similar scenario, Sather et al. (2013) reported that metsulfuron-containing herbicides applied in the summer reduced yield in the fall harvest compared to earlier spring applications, but also resulted in greater yields the following spring. The authors posited that greater root carbohydrate partitioning occurred in response to the summer applications. Even with low leaf area index, carbohydrates can accumulate in tall fescue due to low temperatures, moisture, or nitrogen availability (Brown and Blaser 1965). The same conclusion may be drawn from our study, where carbohydrates accumulated in spring treatments and were converted to new leaf tissue by the time of the summer harvest.

No differences were observed in tall fescue leaf yield when combined across application timing or herbicide rate. The absence of yield reductions provides further evidence that tall fescue had recovered by the summer harvest. Also, by one year after fall applications, no differences were observed in tall fescue tiller density.

**Forage Quality**

No interactions were observed at spring harvest for forage nutritive values, but the main effects of application timing and rate were significant ($P \leq 0.01$). Spring applications resulted in the greatest CP and least ADF, NDF, and lignin (Table 21). Improvements in these forage quality indicators are due to reductions in seedhead and stem proportions of harvested forage. Fiber content of tall fescue increases as tall fescue matures and growth is prioritized to the production of seedheads. Also, cell walls become more lignified as plants mature. Thus, younger leaves are higher quality than older leaves. Tall fescue treated in the fall may have lower quality than tall fescue treated in the spring due to leaf age, but fewer stems improved quality compared to nontreated forage. Averaged across timings, ACP plus metsulfuron increased CP content by 1.1 to 2.5% compared to the nontreated control (Table 22). Higher herbicide rates also led to reduced fiber content, as expected from the lower stem proportions. The minerals Ca, Mg, and P also tended to be greater in forage treated in the spring and also forage receiving higher rates of ACP plus metsulfuron. RFQ is an index of forage quality that takes into account CP, intake, and digestibility and predicts animal performance more accurately than relative feed value (Ball et al. 2001). Regardless of rate, RFQ was highest in early spring treatments, but late fall treatments also resulted in greater RFQ than the nontreated control. When averaged over timings, RFQ increased with ACP plus metsulfuron rate to an index of 120.4 at the highest rate.

At the summer harvest, the main effect of herbicide rate was not significant for CP, ADF, NDF, Ca, and RFQ ($P \geq 0.13$). The main effect of application timing was significant for CP, ADF, NDF, and Ca ($P \leq 0.02$) and early spring treatments had less CP and more ADF and NDF than fall treatments (Table 23). However, these differences were small and application timing did not affect RFQ ($P = 0.11$).

Results of this research indicate ACP plus metsulfuron applied in late fall and early spring reduced tall fescue seedheads, but the late fall application had less of an impact on tall fescue injury and spring harvest yield. Increasing rates of ACP plus metsulfuron also resulted in greater seedhead reduction and yield reduction. Doubling of ACP plus metsulfuron rate as a result of sprayer overlap can cause significant injury to tall fescue, especially when applied in the spring. ACP plus metsulfuron increased CP and reduced ADF and NDF to a greater extent when applied in the spring than in the fall. Increasing ACP plus metsulfuron rate also led to higher CP and less ADF and NDF. Fall applications of ACP plus metsulfuron may be utilized to as part of an alkaloid management plan to reduce the severity of fescue toxicosis with less impact on grass productivity than spring applications.

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# Part 5

# Conclusions

**Part 2**

Metsulfuron applied alone or in combination with ACP or aminopyralid reduced seedhead density and total ergot alkaloid concentrations in spring-harvested tall fescue forage. Forage quality was also greater in all treatments containing metsulfuron due to the low proportion of stems. Animal performance may be improved by consumption of less toxic and more nutritive forage. However, treatments containing metsulfuron also injured tall fescue and reduced yield in the spring. By late summer, tall fescue had recovered, and forage yields and nutritive values were similar. Metsulfuron applied alone or in combination with ACP or aminopyralid may be utilized to reduce the severity of fescue toxicosis with no long-term effects on grass productivity.

**Part 3**

Tall fescue responds differently to ACP plus metsulfuron applied at different times of the year and also different rates. Tall fescue injury was high with spring applications, but recovery was sooner than with fall applications. Fall applications reduced yields the following spring, but also improved forage quality. Tall fescue tiller densities were similar and further suggest long term recovery in tall fescue. Results from these experiments indicate tall fescue is able to recover from aminocyclopyrachlor plus metsulfuron applications, although the extent of injury depends on application timing and rate. Results from this research can help producers predict additional impacts on tall fescue when planning weed control strategies at different times of the year. In addition to weed control, aminocyclopyrachlor plus metsulfuron can improve forage quality of tall fescue, but higher rates applied in the fall may reduce forage yield the following spring.

## Part 4

ACP plus metsulfuron applied in late fall and early spring reduced tall fescue seedheads, but the late fall application had less of an impact on tall fescue injury and spring harvest yield. Increasing rates of ACP plus metsulfuron also resulted in greater seedhead reduction and yield reduction. Doubling of ACP plus metsulfuron rate caused by sprayer overlap can result in significant injury to tall fescue, especially when applied in the spring. ACP plus metsulfuron increased CP and reduced ADF and NDF to a greater extent when applied in the spring than in the fall. Increasing ACP plus metsulfuron rate also led to higher CP and less ADF and NDF. ACP plus metsulfuron may be applied in the fall to suppress tall fescue seedheads with less injury than spring applications and similar improvements to forage quality. Fall applications of ACP plus metsulfuron may be utilized to as part of an alkaloid management plan to reduce the severity of fescue toxicosis with less impact on grass productivity than spring applications.

**Appendices**

**Tables**

Table 1. Herbicide application and harvest dates in 2012 and 2013 field experiments.

| | 1st year | | 2nd year | |
|---|---|---|---|---|
| Activity | Alcoa | Crossville | Alcoa | Crossville |
| Herbicide appl. | Mar. 19, 2012 | Mar. 21, 2012 | Apr. 3, 2013 | Apr. 9, 2013 |
| Spring harvest | Apr. 27, 2012 | May 10, 2012 | May 23, 2013 | May 29, 2013 |
| Summer harvest | Jun. 21, 2012 | Jul. 3, 2012 | Aug. 20, 2013 | Aug. 22, 2013 |
| Year-after harvest | May 16, 2013 | May 29, 2013 | May 13, 2014 | May 27, 2014 |

Table 2. Discoloration and stunting of tall fescue in response to herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | 2 WAT[b] | | 4 WAT | | 8 WAT | | Summer harvest | |
|---|---|---|---|---|---|---|---|---|---|
| | | Discolor-ation[c] | Stunting | Discolor-ation | Stunting | Discolor-ation | Stunting | Discolor-ation | Stunting |
| | g ha$^{-1}$ | --------------------------------- | ------------------ | ------------------ | ------------------ | % | ------------------ | ------------------ | --------------------------------- |
| ACP + MET | 47 + 7 | 11 b | 19 b | 17 b | 38 b | 10 bc | 18 b | 3 b | 6 ab |
| ACP + MET | 78 + 12 | 16 a | 27 a | 23 a | 49 a | 9 c | 24 a | 4 ab | 5 b |
| MET | 7 | 12 b | 21 b | 18 b | 37 b | 7 c | 14 c | 4 ab | 4 b |
| MET | 12 | 15 a | 28 a | 26 a | 53 a | 15 a | 28 a | 5 ab | 8 ab |
| APD + MET | 77 + 12 | 16 a | 29 a | 23 a | 50 a | 13 ab | 24 a | 6 a | 8 a |
| ACP + 2,4-D | 78 + 591 | 0 c | 0 c | 0 c | 0 c | 0 d | 0 d | 0 c | 0 c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Abbreviations: WAT, weeks after treatment; ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.
[c] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$. All data were arcsin transformed prior to analysis to improve normality. Nontransformed means are presented for clarity.

Table 3. Tall fescue seedhead density, yield, and total ergot alkaloid concentration at spring harvest in response to clipping and herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | Fescue Seedheads[b] | | Total Biomass Yield | | Fescue Leaves | | Fescue Stems | | Other | | Alkaloid Concentration | | Fescue Tillers | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g ha$^{-1}$ | no. m$^{-2}$ | | ----------------------------kg ha$^{-1}$---------------------------- | | | | | | | | µg kg$^{-1}$ | | no. m$^{-2}$ | |
| ACP + MET[c] | 47 + 7 | 149 | c | 2526 | bc | 640 | b | 656 | b | 1522 | ab | 1074 | cd | 753 | c |
| ACP + MET | 78 + 12 | 143 | c | 2010 | c | 568 | b | 603 | b | 1165 | c | 983 | d | 870 | abc |
| MET | 7 | 202 | c | 2671 | b | 724 | b | 996 | b | 1150 | c | 1091 | cd | 794 | bc |
| MET | 12 | 158 | c | 2251 | bc | 575 | b | 578 | b | 1147 | c | 1022 | cd | 878 | abc |
| APD + MET | 77 + 12 | 150 | c | 2180 | bc | 542 | b | 569 | b | 1309 | abc | 978 | d | 793 | bc |
| ACP + 2,4-D | 78 + 591 | 399 | a | 4275 | a | 1225 | a | 2171 | a | 1368 | abc | 1771 | a | 1024 | a |
| Clip | -- | 183 | c | 3785 | a | 1095 | a | 1020 | b | 1243 | bc | 1298 | bc | 1004 | a |
| Nontreated | -- | 317 | b | 4139 | a | 1090 | a | 1726 | a | 1591 | a | 1484 | b | 936 | ab |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v. Clipping treatment was applied two weeks before first harvest.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.
[c] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.

Table 4. Yield and total ergot alkaloid concentration in tall fescue at summer harvest in response to clipping and herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | Total Biomass Yield[b] | | Fescue Leaves | | Alkaloid Concentration | | Fescue Tillers | |
|---|---|---|---|---|---|---|---|---|---|
| | $g\ ha^{-1}$ | ------------$kg\ ha^{-1}$------------ | | | | $\mu g\ kg^{-1}$ | | no. $m^{-2}$ | |
| ACP + MET[c] | 47 + 7 | 1869 | a | 1513 | a | 828 | a | 763 | a |
| ACP + MET | 78 + 12 | 1805 | a | 1840 | a | 869 | a | 876 | a |
| MET | 7 | 2027 | a | 1570 | a | 760 | a | 845 | a |
| MET | 12 | 1931 | a | 1435 | a | 804 | a | 914 | a |
| APD + MET | 77 + 12 | 2003 | a | 1278 | a | 673 | a | 751 | a |
| ACP + 2,4-D | 78 + 591 | 1773 | a | 1856 | a | 713 | a | 869 | a |
| Clip | -- | 1836 | a | 1500 | a | 768 | a | 896 | a |
| Nontreated | -- | 1650 | a | 1411 | a | 701 | a | 755 | a |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v. Clipping treatment was applied two weeks before first harvest.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.
[c] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.

Table 5. Yield and tiller density of tall fescue at year-after harvest in response to clipping and herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | Total Biomass Yield[b] | | Fescue Tillers | |
|---|---|---|---|---|---|
| | g $ha^{-1}$ | kg $ha^{-1}$ | | no. $m^{-2}$ | |
| ACP + MET[c] | 47 + 7 | 4179 | a | 897 | a |
| ACP + MET | 78 + 12 | 4274 | a | 875 | a |
| MET | 7 | 4118 | a | 903 | a |
| MET | 12 | 4211 | a | 861 | a |
| APD + MET | 77 + 12 | 3902 | a | 780 | a |
| ACP + 2,4-D | 78 + 591 | 4276 | a | 891 | a |
| Clip | -- | 4162 | a | 832 | a |
| Nontreated | -- | 3960 | a | 866 | a |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v. Clipping treatment was applied two weeks before first harvest.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.
[c] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.

Table 6. Tall fescue nutritive values at spring harvest in response to clipping and herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | CP[b,c] | | ADF | | NDF | | Lignin | | Ca | | Mg | | RFQ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $g\ ha^{-1}$ | ---------------------------------------------- | | | | | | % | | | | ---------------------------------------------- | | -- | |
| ACP + MET | 47 + 7 | 14.23 | bc | 34.31 | b | 64.62 | b | 4.703 | abc | 0.216 | bcd | 0.204 | bc | 105.48 | ab |
| ACP + MET | 78 + 12 | 15.34 | a | 33.15 | d | 63.06 | c | 4.486 | c | 0.241 | a | 0.218 | a | 107.57 | a |
| MET | 7 | 14.52 | bc | 34.24 | bc | 64.23 | bc | 4.548 | bc | 0.236 | ab | 0.212 | ab | 105.30 | ab |
| MET | 12 | 14.90 | ab | 33.53 | cd | 63.53 | bc | 4.483 | c | 0.238 | ab | 0.215 | ab | 107.55 | a |
| APD + MET | 77 + 12 | 14.68 | ab | 33.85 | bcd | 64.18 | bc | 4.679 | abc | 0.229 | abc | 0.208 | abc | 105.67 | ab |
| ACP + 2,4-D | 78 + 591 | 13.21 | d | 36.29 | a | 66.71 | a | 4.937 | a | 0.201 | d | 0.195 | c | 99.03 | c |
| Clip | -- | 13.89 | c | 35.52 | a | 66.17 | a | 4.807 | ab | 0.210 | cd | 0.208 | abc | 98.56 | c |
| Nontreated | -- | 13.20 | d | 35.91 | a | 65.94 | a | 4.953 | a | 0.210 | cd | 0.197 | c | 102.21 | bc |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v. Clipping treatment was applied two weeks before first harvest.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.
[c] Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality; ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.

Table 7. Tall fescue nutritive values at summer harvest in response to clipping and herbicide treatments across four site-years in Tennessee.

| Treatment[a] | Rate | CP[b,c] | | ADF | | NDF | | RFQ | |
|---|---|---|---|---|---|---|---|---|---|
| | g $ha^{-1}$ | -----------------------%----------------------- | | | | | | -- | |
| ACP + MET[c] | 47 + 7 | 11.40 | a | 36.78 | a | 68.64 | a | 97.35 | a |
| ACP + MET | 78 + 12 | 11.40 | a | 36.46 | a | 68.36 | a | 97.87 | a |
| MET | 7 | 11.36 | a | 36.88 | a | 68.90 | a | 96.05 | a |
| MET | 12 | 11.24 | a | 36.58 | a | 68.52 | a | 97.72 | a |
| APD + MET | 77 + 12 | 11.04 | a | 37.42 | a | 69.90 | a | 93.45 | a |
| ACP + 2,4-D | 78 + 591 | 11.47 | a | 36.67 | a | 69.30 | a | 96.27 | a |
| Clip | -- | 10.84 | a | 37.08 | a | 69.00 | a | 95.21 | a |
| Nontreated | -- | 10.78 | a | 37.30 | a | 69.22 | a | 94.80 | a |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v. Clipping treatment was applied two weeks before first harvest.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.
[c] Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality; ACP, aminocyclopyrachlor; MET, metsulfuron; APD, aminopyralid.

Table 8. Herbicide application dates at four timings in 2012 and 2013 field experiments.

| | 2012 | | 2013 | |
|---|---|---|---|---|
| Timing | Alcoa | Crossville | Alcoa | Crossville |
| Spring | Mar. 19 | Mar. 21 | Apr. 3 | Apr. 9 |
| Summer | Jun. 25 | Jun. 28 | Jun. 21 | Jul. 8 |
| Early fall | Sep. 19 | Sep. 24 | Sep. 18 | Sep. 26 |
| Late fall | Nov. 20 | Nov. 28 | Dec. 20 | Dec. 19 |

Table 9. Tall fescue discoloration and stunting in response to four application timings and three rates of aminocyclopyrachlor plus metsulfuron across four site-years in Tennessee.

| | | 2 WAT | | 4 WAT | | 8 WAT | |
|---|---|---|---|---|---|---|---|
| Timing[a] | ACP + MET[b] rate | Discolor-ation[c] | Stunting | Discolor-ation | Stunting | Discolor-ation | Stunting |
| | g ha$^{-1}$ | ---% | | | | | --- |
| Spring | 47 + 7 | 11 e | 21 cd | 16 e | 37 c | 8 f | 14 def |
| | 78 + 12 | 16 d | 27 b | 21 cde | 45 b | 13 def | 21 c |
| | 155 + 23 | 25 a | 37 a | 29 b | 60 a | 18 c | 33 ab |
| Summer | 47 + 7 | 15 de | 17 de | 16 e | 23 ef | 11 ef | 13 ef |
| | 78 + 12 | 20 abc | 19 cde | 22 cd | 29 d | 9 f | 15 de |
| | 155 + 23 | 22 ab | 24 bc | 35 a | 41 bc | 15 cde | 21 c |
| Early fall | 47 + 7 | 12 de | 11 f | 20 de | 20 f | 19 c | 19 cd |
| | 78 + 12 | 16 cd | 14 ef | 26 bc | 27 de | 27 b | 28 b |
| | 155 + 23 | 20 bc | 18 de | 36 a | 36 c | 36 a | 38 a |
| Late fall | 47 + 7 | 2 fg | 2 g | 3 fg | 3 gh | 8 f | 6 g |
| | 78 + 12 | 3 fg | 2 g | 5 fg | 4 gh | 12 def | 9 fg |
| | 155 + 23 | 6 f | 5 g | 8 f | 7 g | 15 cd | 12 ef |
| Nontreated | -- | 0 g | 0 g | 0 g | 0 h | 0 g | 0 h |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; WAT, weeks after treatment.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 10. Tall fescue discoloration and stunting at spring harvest in response to two application timings of aminocyclopyrachlor plus metsulfuron across four site-years in Tennessee.

| Timing[a,b] | Discolor-ation[c] | Stunting |
|---|---|---|
| | % | |
| Early fall | 0 b | 4 b |
| Late fall | 7 a | 13 a |
| Nontreated | 0 b | 0 c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, and 155 + 23 g $ha^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 11. Tall fescue discoloration and stunting at spring harvest in response to three application rates of aminocyclopyrachlor plus metsulfuron across four site-years in Tennessee.

| Treatment[a,b] | Rate | Discolor-ation[c] | | Stunting | |
|---|---|---|---|---|---|
| | g $ha^{-1}$ | % | | | |
| ACP + MET[d] | 47 + 7 | 3 | b | 4 | b |
| ACP + MET | 78 + 12 | 3 | b | 6 | b |
| ACP + MET | 155 + 23 | 5 | a | 13 | a |
| Nontreated | -- | 0 | c | 0 | c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across early fall and late fall application timings.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.
[d] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron.

Table 12. Horsenettle control with summer-applied aminocyclopyrachlor plus metsulfuron at three rates across two site years in Tennessee.

| Treatment[a] | Rate | 4 WAT[b,c] | | 8 WAT | |
|---|---|---|---|---|---|
| | g ha$^{-1}$ | % | | | |
| ACP + MET | 47 + 7 | 83 | b | 96 | b |
| ACP + MET | 78 + 12 | 87 | b | 95 | b |
| ACP + MET | 155 + 23 | 96 | a | 99 | a |
| Nontreated | -- | 0 | c | 0 | c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Means within a column followed by the same letter are not statistically different according to Fisher's Protected LSD at $P \leq 0.05$. Data were collected from two year-location combinations and were arcsin square-root transformed prior to analysis to improve normality. Nontransformed means are presented for clarity.
[c] Abbreviations: WAT, weeks after treatment; ACP, aminocyclopyrachlor; MET, metsulfuron.

Table 13. Tall fescue tiller density and yield in response to four application timings and three rates of aminocyclopyrachlor plus metsulfuron across four site-years in Tennessee.

| | | 6 MAIT | 14 MAIT | | |
|---|---|---|---|---|---|
| Timing[a] | ACP + MET[b] rate | Fescue Tillers | Total Biomass Yield[c] | | Fescue Tillers |
| | g ha$^{-1}$ | no. m$^{-2}$ | kg ha$^{-1}$ | | no. m$^{-2}$ |
| Spring | 47 + 7 | 713 | 3797 | abc | 832 |
| | 78 + 12 | 624 | 3962 | ab | 920 |
| | 155 + 23 | 669 | 3936 | ab | 803 |
| Summer | 47 + 7 | 750 | 4022 | a | 883 |
| | 78 + 12 | 805 | 4093 | a | 954 |
| | 155 + 23 | 698 | 4037 | a | 846 |
| Early fall | 47 + 7 | | 3717 | abcd | 802 |
| | 78 + 12 | | 3592 | bcd | 735 |
| | 155 + 23 | | 3401 | d | 798 |
| Late fall | 47 + 7 | | 3839 | abc | 887 |
| | 78 + 12 | | 3533 | cd | 835 |
| | 155 + 23 | | 3009 | e | 785 |
| Nontreated | -- | 765 | 4001 | a | 910 |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; MAIT, months after initial treatment.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 14. Tall fescue nutritive values at spring harvest in response to four timings of aminocyclopyrachlor plus metsulfuron applied in previous year across four site-years in Tennessee.

| Timing[a,b] | CP[c,d] | | ADF | | NDF | | Lignin | | RFQ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ---------------------------------%--------------------------------- | | | | | | | | -- | |
| Spring | 12.26 | c | 35.05 | b | 63.11 | b | 4.04 | bc | 110.60 | b |
| Summer | 11.94 | cd | 35.39 | ab | 63.47 | ab | 4.20 | ab | 109.89 | b |
| Early fall | 12.78 | b | 34.09 | c | 61.96 | c | 3.94 | cd | 114.85 | a |
| Late fall | 13.21 | a | 33.94 | c | 61.35 | c | 3.86 | d | 115.74 | a |
| Nontreated | 11.54 | d | 36.09 | a | 64.32 | a | 4.36 | a | 106.35 | c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, and 155 + 23 g $ha^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.
[d] Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality.

Table 15. Herbicide application dates at two timings and harvest dates from field experiments in 2012-2014.

| | 1st year | | 2nd year | |
|---|---|---|---|---|
| Activity | Alcoa | Crossville | Alcoa | Crossville |
| Late fall appl. | Nov. 20, 2012 | Nov. 28, 2012 | Dec. 3, 2013 | Dec. 4, 2013 |
| Early spring appl. | Apr. 3, 2013 | Apr. 9, 2013 | Mar. 20, 2014 | Apr. 1, 2014 |
| Spring harvest | May 24, 2013 | May 28, 2013 | May 20, 2014 | May 30, 2014 |
| Summer harvest | Jul. 30, 2013 | Aug. 21, 2013 | Aug 21, 2014 | Aug. 28, 2014 |

Table 16. Discoloration and stunting in tall fescue in response to aminocyclopyrachlor plus metsulfuron applied in late fall and early spring across four site-years in Tennessee.

| | | 2 WAT | | 4 WAT | | 8 WAT | Spring Harvest | |
|---|---|---|---|---|---|---|---|---|
| Timing[a] | ACP + MET[b] rate | Discoloration[c] | Stunting | Discoloration | Stunting | Stunting | Discoloration | Stunting |
| | g ha$^{-1}$ | % | | | | | | |
| Late fall | 47 + 7 | 4 e | 2 fg | 6 e | 4 f | 6 e | 1 d | 4 f |
| | 78 + 12 | 5 e | 2 efg | 6 e | 4 f | 9 e | 1 d | 10 e |
| | 93 + 14 | 5 e | 3 ef | 8 de | 7 ef | 11 de | 2 cd | 13 e |
| | 155 + 23 | 7 e | 5 e | 11 cd | 9 e | 14 cd | 3 cd | 20 d |
| Early spring | 47 + 7 | 11 d | 15 d | 12 c | 27 d | 18 c | 5 c | 18 d |
| | 78 + 12 | 15 c | 22 c | 19 b | 37 c | 29 b | 10 b | 29 c |
| | 93 + 14 | 19 b | 27 b | 22 b | 43 b | 33 b | 13 ab | 33 b |
| | 155 + 23 | 23 a | 32 a | 31 a | 53 a | 42 a | 16 a | 42 a |
| Nontreated | -- | 0 f | 0 g | 0 f | 0 g | 0 f | 0 d | 0 f |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; WAT, weeks after treatment.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 17. Effects of aminocyclopyrachlor plus metsulfuron application timing on tall fescue leaf and stem yield, other species yield, and tall fescue tiller density at spring harvest across four site-years in Tennessee.

| Timing[a,b] | Fescue Leaves[c] | | Fescue Stems | Other | | Fescue Tillers[d] |
|---|---|---|---|---|---|---|
| | --------------------kg $ha^{-1}$-------------------- | | | | | no. $m^{-2}$ |
| Late fall | 1292 | a | 824 | 2140 | a | 660 |
| Early spring | 714 | b | 700 | 1753 | b | 699 |
| Nontreated | 1308 | a | 1945 | 2364 | a | 705 |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g $ha^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.
[d] Tiller density was determined two weeks after spring harvest.

Table 18. Effects of aminocyclopyrachlor plus metsulfuron application rate on tall fescue leaf and stem yield, other species yield, and tall fescue tiller density at spring harvest across four site-years in Tennessee.

| Treatment[a,b] | Rate | Fescue Leaves | Fescue Stems[c] | | Other | Fescue Tillers[d] | |
|---|---|---|---|---|---|---|---|
| | $g\ ha^{-1}$ | --------------------- | $kg\ ha^{-1}$ | --------------------- | | $no.\ m^{-2}$ | |
| ACP + MET[e] | 47 + 7 | 1177 | 1156 | b | 1881 | 754 | a |
| ACP + MET | 78 + 12 | 937 | 748 | c | 2104 | 678 | ab |
| ACP + MET | 93 + 14 | 1008 | 667 | c | 1873 | 676 | ab |
| ACP + MET | 155 + 23 | 888 | 478 | c | 1929 | 605 | b |
| Nontreated | -- | 1308 | 1945 | a | 2364 | 705 | ab |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across late fall and early spring application timings.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.
[d] Tiller density was determined two weeks after spring harvest.
[e] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron

Table 19. Effects of aminocyclopyrachlor plus metsulfuron application timing on tall fescue summer harvest yield and fall tiller density across four site-years in Tennessee.

| Timing[a,b] | Total Biomass Yield[c] | | Fescue Leaves | Fescue Tillers[d] |
|---|---|---|---|---|
| | ------------kg ha$^{-1}$------------ | | | no. m$^{-2}$ |
| Late fall | 1598 | b | 1679 | 638 |
| Early spring | 1929 | a | 1533 | 632 |
| Nontreated | 1559 | b | 1293 | 640 |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g ha$^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.
[d] Tiller density was determined one year after late fall herbicide application.

Table 20. Effects of aminocyclopyrachlor plus metsulfuron application rate on tall fescue summer harvest yield and fall tiller density across four site-years in Tennessee.

| Treatment[a,b] | Rate | Total Biomass Yield | Fescue Leaves | Fescue Tillers[c] |
|---|---|---|---|---|
| | g ha$^{-1}$ | ------------kg ha$^{-1}$ | ------------ | no. m$^{-2}$ |
| ACP + MET[d] | 47 + 7 | 1825 | 1604 | 647 |
| ACP + MET | 78 + 12 | 1763 | 1667 | 640 |
| ACP + MET | 93 + 14 | 1775 | 1735 | 605 |
| ACP + MET | 155 + 23 | 1691 | 1418 | 648 |
| Nontreated | -- | 1559 | 1293 | 640 |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across late fall and early spring application timings.
[c] Tiller density was determined one year after late fall herbicide application.
[d] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron.

Table 21. Effects of aminocyclopyrachlor plus metsulfuron application timing on tall fescue nutritive values at spring harvest across four site-years in Tennessee.

| Timing[a,b] | CP[c,d] | | ADF | | NDF | | Lignin | | Ca | | Mg | | P | | RFQ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ----------------------------------------------------- | | | | | | %----------------------------------------------------- | | | | | | | | -- | |
| Late fall | 11.8 | b | 34.5 | b | 62.3 | b | 3.88 | b | 0.378 | b | 0.245 | b | 0.241 | b | 113.93 | b |
| Early spring | 12.9 | a | 33.7 | c | 61.3 | c | 3.70 | c | 0.400 | a | 0.261 | a | 0.245 | a | 117.34 | a |
| Nontreated | 10.6 | c | 36.6 | a | 64.4 | a | 4.43 | a | 0.371 | b | 0.238 | b | 0.231 | c | 104.98 | c |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g $ha^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality.
[d] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 22. Effects of aminocyclopyrachlor plus metsulfuron application rate on tall fescue nutritive values at spring harvest across four site-years in Tennessee.

| ACP + MET rate[a,b,c] | CP[d] | | ADF | | NDF | | Lignin | | Ca | | Mg | | P | | RFQ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g ha$^{-1}$ | ---------------------------------------------------%--------------------------------------------------- | | | | | | | | | | | | | | -- | |
| 47 + 7 | 11.7 | c | 35.0 | b | 62.9 | b | 4.03 | b | 0.368 | d | 0.244 | cd | 0.238 | c | 112.2 | c |
| 78 + 12 | 12.2 | b | 34.3 | c | 62.0 | c | 3.81 | c | 0.389 | b | 0.255 | ab | 0.243 | b | 114.7 | bc |
| 93 + 14 | 12.3 | b | 34.1 | c | 61.9 | c | 3.82 | c | 0.384 | bc | 0.251 | bc | 0.243 | b | 115.4 | b |
| 155 + 23 | 13.1 | a | 33.0 | d | 60.4 | d | 3.51 | d | 0.415 | a | 0.262 | a | 0.249 | a | 120.4 | a |
| Nontreated | 10.6 | d | 36.6 | a | 64.4 | a | 4.43 | a | 0.371 | cd | 0.238 | d | 0.231 | d | 105.0 | d |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across late fall and early spring application timings.
[c] Abbreviations: ACP, aminocyclopyrachlor; MET, metsulfuron; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality.
[d] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

Table 23. Effects of aminocyclopyrachlor plus metsulfuron application timing on tall fescue nutritive values at summer harvest across four site-years in Tennessee.

| Timing[a,b] | CP[c,d] | | ADF | | NDF | | Ca | | RFQ |
|---|---|---|---|---|---|---|---|---|---|
| | ---------------------------------- | | | | % | | ---------------------------------- | | -- |
| Late fall | 12.8 | a | 34.1 | b | 61.1 | b | 0.477 | b | 112.4 |
| Early spring | 12.4 | b | 34.8 | a | 62.5 | a | 0.440 | c | 110.7 |
| Nontreated | 12.7 | ab | 33.8 | b | 59.6 | c | 0.509 | a | 113.1 |

[a] Herbicide treatments were applied with non-ionic surfactant at 0.25% v/v.
[b] Data averaged across application rates of 47 + 7, 78 + 12, 93 + 14, and 155 + 23 g $ha^{-1}$ aminocyclopyrachlor plus metsulfuron.
[c] Abbreviations: CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RFQ, relative forage quality.
[d] Means within a column followed by the same letter are not statistically different according to the *t* test on difference of least square means at $P \leq 0.05$.

## Figures

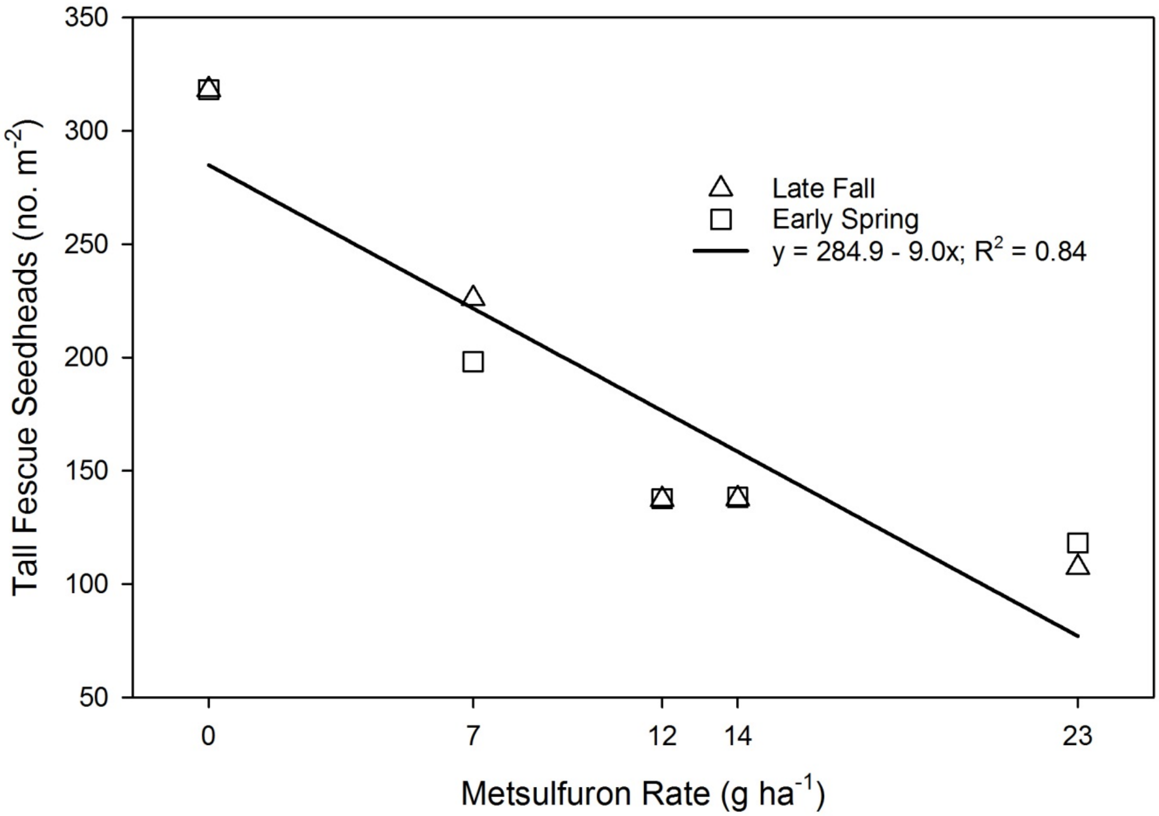


Figure 1. Seedhead density of tall fescue treated with metsulfuron at 0, 7, 12, 14, and 23 g ha$^{-1}$ in the late fall or early spring. Each treatment included 6.6 g aminocyclopyrachlor per g of metsulfuron and non-ionic surfactant at 0.25% v/v. Values are averages of four site-years in Tennessee. Slope values were not different (P = 0.76) for the late fall and early spring application timings as determined by analysis of covariance; therefore, the regression slope was fitted across both timings.

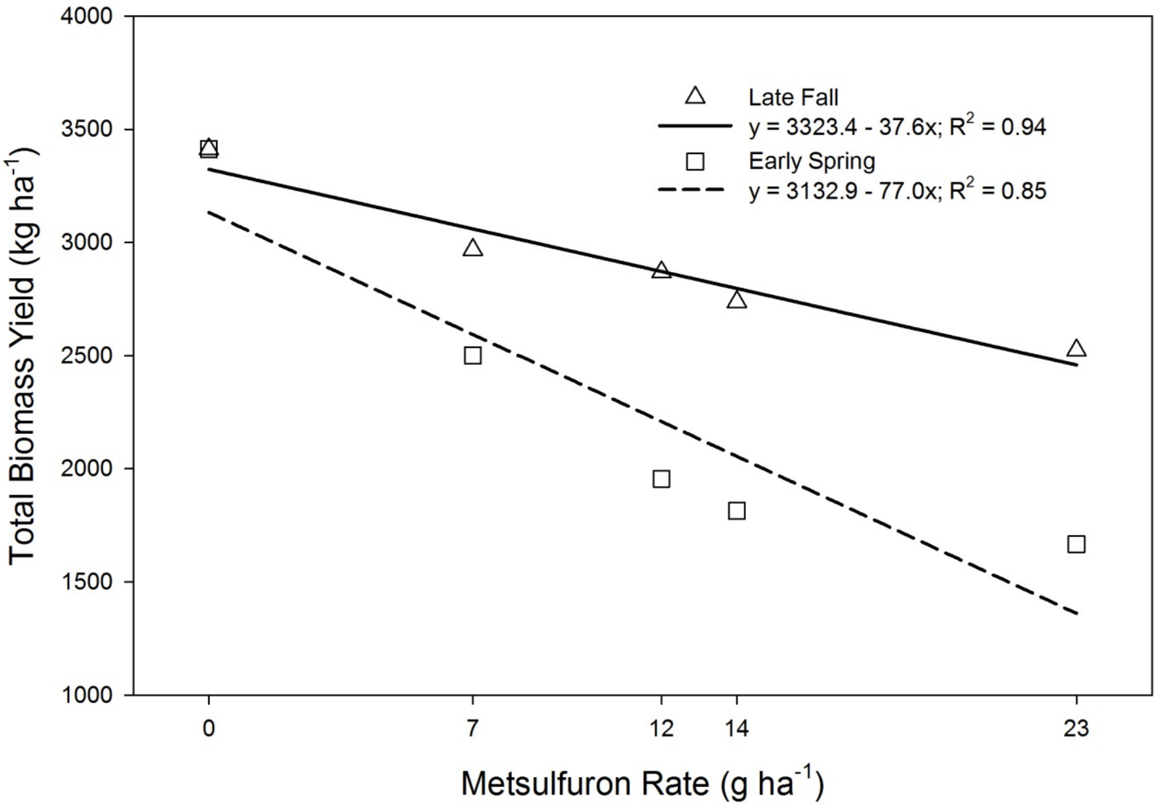


Figure 2. Total biomass yield at spring harvest of tall fescue treated with metsulfuron at 0, 7, 12, 14, and 23 g ha$^{-1}$ in the late fall or early spring. Each treatment included 6.6 g aminocyclopyrachlor per g of metsulfuron and non-ionic surfactant at 0.25% v/v. Values are averages of four site-years in Tennessee. Slope values differed ($P < 0.001$) between lines as determined by analysis of covariance.

## Vita

Trevor Davis Israel was born November 12, 1985 to Lonnie and Gail Israel of Candler, NC. Trevor was exposed to plants at an early age and grew up helping his family at their garden center in Asheville, NC. He has always maintained a love for the outdoors and achieved the rank of Eagle Scout in the Boy Scouts of America. He graduated from Enka High School in 2003 and went on to pursue an engineering degree in college. However, Trevor decided that a career in the natural sciences would be more fulfilling and received and Bachelor of Science degree in Environmental Sciences with a concentration in Watershed Hydrology from North Carolina State University in 2008. After working for the Aquatic Weed Control Program in the North Carolina Division of Water Resources, Trevor seized an opportunity to pursue graduate studies in aquatic herbicide/plant interactions with Dr. Rob Richardson at North Carolina State University. He received a Master of Science degree in Crop Science with a concentration in Weed Science in 2011. After a successful visit to the University of Tennessee, Trevor accepted a position as Extension Assistant with Dr. Neil Rhodes to develop the Herbicide Stewardship Program and conduct weed research trials in forages. In addition, Trevor also began work toward a Doctor of Philosophy degree in Plants, Soils, and Insects with a concentration in Weed Science. Trevor has presented at numerous extension and professional meetings, winning awards for oral and poster competitions. To date, Trevor has authored 1 peer-reviewed journal article and 16 extension publications. Following completion of his doctorate degree, Trevor will begin a new career as an industry professional.