REDUCING ERGOVALINE AND ERGOT ALKALOID CONCENTRATIONS THROUGH FERTILIZER, HERBICIDE AND CLIPPING MANAGEMENT

A Dissertation
Presented to
The Faculty of the Graduate School
University of Missouri – Columbia

In Partial Fulfillment
Of the Requirements for the Degree

Doctor of Philosophy

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by
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MAY 2010

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REDUCING ERGOVALINE AND ERGOT ALKALOID CONCENTRATIONS THROUGH FERTILIZER, HERBICIDE AND CLIPPING MANAGEMENT

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This dissertation is dedicated to my parents, Clyde and Linda Rogers. If I had not had their support and encouragement over the years, everything would have been very difficult.

ACKNOWLEDGEMENTS

I would like to thank Dr. Craig Roberts for providing the opportunity for me to obtain a doctoral degree.

I would like to thank the following for their hard work and support: Ryan Lock, Heather Benedict, Patrick Teoh, Ying Ying, Matt Massie, Dr. Dave Davies, Forage Systems Research Center, Linneus, MO, Valerie Tate, Forage Systems Research Center, Linneus, MO, Dr. Dale Blevins, Dr. Will McClain, Dr. Robert Kallenbach, Dr. George Rottinghaus, Dr. Donald Spiers, Dr. Nick Hill, University of Georgia, Kimberly Hill, Agrinostics, Ltd. Dr. John Andrea, Clemson University, Greg Durham, University of Georgia, Elizabeth Hamilton, Dr. Abner Womack, Food and Agriculture Research Institute, University of Missouri (FAPRI), Dr. Scott Brown, FAPRI, Brent Carpenter, FAPRI, Melvin Brees, FAPRI, Dr. Pat Guinan, University of Missouri State Extension Climatologist, Dr. George Justice, Vice Provost for Advanced Studies and Dean of the Graduate School and University of Missouri's Interlibrary Borrowing Office (ILL@MU). I would like to thank my former FAPRI supervisor, Dr. Verl Benson and my coworkers, in particular Dr. Todd Farrand, Dr. Walaiporn Intarapapong and Judy Richards for their support and encouragement throughout the doctoral process. Thank you to Dr. Norma Fisk, Jan Lysaght and Julie Barry for their priceless assistance with the dissertation, defense and life in general.

I would like to thank the Columbia, MO Tai Chi Chuan community for their support, encouragement and their assistance in maintaining my sanity, including (in no particular order), Pablo Mendoza, Kenny Greene, Brandon Smith, Dr. Sandy Matsuda, Gladys Swan, Micheal Crist, Charles Baerwald, Dr. W. 'Kal' Johnson, John Gage and Dale Langford. I think of everyone in the Tai Chi Chuan community as my teachers. Thank you, for taking this journey with me.

If you feel I have forgotten to thank you by name, I mean no disrespect. I recognize this dissertation project would not have been possible without the assistance of many people.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF EQUATIONS

REDUCING ERGOVALINE AND ERGOT ALKALOID CONCENTRATIONS THROUGH FERTILIZER, HERBICIDE AND CLIPPING MANAGEMENT

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ABSTRACT

Animals grazing tall fescue grass (*Lolium arundinaceum*) infected with *Neotyphodium coenophialum* consume the toxins ergovaline and other ergot alkaloids. This research included a series of experiments with two overall objectives: 1) to explore management practices that might reduce alkaloid concentration and 2) to estimate if change in management was economically feasible. Three experiments in this study resulted in reduced ergovaline concentration. The first experiment showed use of poultry litter rather than chemical NPK reduced ergovaline by at least 124 µg kg^{-1} DM. Another experiment showed the herbicide clethodim reduced ergovaline up to 72%. A third experiment, conducted with Clemson University and the University of Georgia, showed that monthly clipping of tall fescue reduced ergovaline in the Spring to the point of partial alkaloid suppression. Economic analysis estimated that clethodim-treated forage would produce the highest calving rates and stocker gains and was the most economically beneficial of all practices studied.

Chapter 1: Introduction and Literature Review

This dissertation explores the effect of fertilizer, clipping and herbicide management on ergot alkaloid and ergovaline concentrations; these alkaloids are present in 'Kentucky-31' tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] infected with the fungal endophyte *Neotyphodium coenophialum* [(Morgan-Jones and Gams) Glenn, Bacon, and Hanlin comb. nov.]. They cause a disorder in livestock called fescue toxicosis. It is hoped that this dissertation will provide cattle producers in the fescue belt information that allow them to make management decisions that will increase herd productivity and farm profitability.

The first chapter of the dissertation is a literature review. It first introduces fescue toxicosis then then discusses the economic impact of fescue toxicosis on cattle fertility. It examines fescue toxicosis effects on livestock and reviews the effects that nitrogen (N) and phosphorus (P) in conventional fertilizer and in poultry litter have on alkaloid concentrations as related to fescue toxicosis, tall fescue and *Neotyphodium*. The review explains the effect of the herbicide clethodim on endophyte-infected tall fescue and possibly why ergovaline and ergot alkaloids are affected by the herbicide. It also discusses the impact that limestone application has on conditions associated with fescue toxicosis, ergot alkaloid and ergovaline concentrations in tall fescue. It explores the literature that describes the structure and composition of ergot alkaloids and ergovaline. The review finishes with a discussion of the fluctuation of ergovaline and ergot alkaloid concentrations in tall fescue through the growing season.

The next six chapters report on various research. Chapter 2 reports the effect of the herbicide clethodim on ergovaline and ergot alkaloid concentrations in tall fescue. Chapter 3 presents the effect of poultry litter and equivalent chemical N-P-potassium (K) fertilizer on ergovaline and ergot alkaloid concentrations in tall fesuce. Chapter 4 explores the effect of P fertilization on ergovaline and ergot alkaloid concentrations in tall fescue. Chapter 5 reports the lack of affect of limestone application on ergovaline and ergot alkaloid concentrations in tall fesuce. Chapter 6 investigates the effect of monthly clipping of tall fescue during a growing season on ergovaline and ergot alkaloid concentration. Chapter 7 reports on ergovaline and ergot alkaloid concentrations in tall fescue that accumulated through a calendar year.

The final research chapter, Chapter 8, is an economic analysis. It predicts the effect of the above management practices on the estimated reduction in average daily gain and calving rate; these reductions assumed to be due to ergovaline concentrations.

The final chapter, Chapter 9, summarizes the findings of the research chapters. This summary chapter suggests several immediate applications for cattle producers that are products of the dissertation research.

Literature Review

Economics of Fescue Toxicosis

The economic impact of fescue toxicosis is due to the effect of toxic, endophyte-infected tall fescue on animal performance. In a survey of the economic impact of endophyte-infected tall fescue, Hoveland (1993) estimated the loss of reduced conception rate for 8.5 million beef cows that grazed tall fescue in 21 states. The economic impact

related to reduced conception rate and totaled an estimated $354 million y^{-1} (Hoveland, 1993). Impact also related to lower ADG, which has a negative economic impact on farm income.

Prior to the establishment of procedures that directly measured ergot alkaloid (Hill and Agee, 1994) and ergovaline (Rottinghaus et al., 1991), daily gain was related to endophyte-infection level of a pasture. Average daily gain is often studied by comparing endophyte-infected and noninfected tall fescue.

In these studies, researchers have reported a linear relationship between endophyte-level and steer ADG. Early workers, such as Stuedemann et al (1985) and Crawford et al (1989) reported such a relationship in pure stands of tall fescue. In addition, Thompson et al. (1993) estimated steer ADG with a linear equation, represented by ADG minus a weight loss multiplied by the percent of a tall fescue pasture's endophyte-infection; their work considered six ADG reduction equations for spring, summer and spring + summer, and included pastures with or without clover. Their equations were developed from combined analyses of observations in 12 tall fescue grazing studies. Beck et al. (2008) then estimated ADG reduction using the Thompson et al. (1993) equation for steers grazing tall fescue-clover in the spring. Estimated ADG reduction corresponded to the observed, 3-y ADG mean reduction in steers grazing 'Kentucky-31' tall fescue compared to a novel endophyte-infected tall fescue (Beck et al., 2008). In addition to these researchers, Fribourg et al. (1991) related endophyte level to steer ADG and showed that a curvilinear relationship resulted when pastures of endophyte-infected tall fescue contained clover (Fribourg et al., 1991).

Based on these studies, there can be little doubt that steer performance can be predicted from the toxic endophyte-level of a pasture. The degree of steer ADG reduction has been estimated by Stuedemann et al. (1985) and Crawford et al. (1989) to be approximately 45 g d^{-1} for each 10% increase in endophyte level.

Besides ADG, calving rate also has been shown to be influenced by the presence of toxic endophyte. In cattle, reduced fertility due to fescue toxicosis has the potential to cause a loss in calf crop (Short et al., 1990). Reductions in conception rates in cows suffering from fescue toxicosis were estimated to be 3.5% for every 10% of endophyte-infection in tall fescue (Porter and Thompson, 1992). According to a survey by Hoveland (1993), reduced conception rates resulted in an estimated 885,000 unborn calves y^{-1} when those cows were grazing toxic tall fescue. In this report, calving rate of cows maintained on tall fescue averaged 74%, compared to 90% in cows grazing non-toxic forage.

Cows consuming toxic tall fescue have failed to become pregnant because of exposure to ergot alkaloids such as ergovaline (Short et al., 1990). Reduced fertility is a physiological response to ergot alkaloids that occurs because of reduced blood to internal reproductive organs. Reduced fertility can also be the result of hormone imbalances or disruptions due to ergot alkaloid exposure (review by Porter and Thompson, 1992). Predicting the economic loss due to reduced fertility would help cattle producers make management decisions that would pay for costs of alleviating fescue toxicosis.

Physiological or Clinical Signs

Physiological or clinical signs of ergot alkaloid poisoning, or fescue toxicosis, include peripheral vasoconstriction, which restricts blood flow to extremities (Oliver,

1997; Strickland et al., 1993; Clark et al., 1978). Poor thermoregulation, most notably heat stress, is observed as animals experience vasoconstriction; this is because reduced peripheral blood flow does not allow vessels and capillaries from the animal's body core to transport warm blood to the animal's surface to cool.

In one study, physical signs of fescue toxicosis were detected in Angus calves weighing 180 to 250 kg and fed a ration containing as little as 50 µg ergovaline $kg^{-1}$and exposed to air temperatures above 32°C (Cornell et al., 1990). In another study, crossbred heifers fed a feed concentrate containing 390 and 790 µg ergovaline kg^{-1} DM and exposed to an ambient air temperature of 7.6 ± 2.9 °C had reduced artery area within 27 h after initial ergovaline exposure (Aiken et al., 2009). In another study, physiological signs of vasoconstriction were measured in heifers within 4 h after feeding a concentrate containing endophyte-infected tall fescue seed (Aiken et al., 2007).

In the summer, heat stress can alter grazing behavior; thus, reducing cattle productivity. Elevated core temperature in a study resulting from of a 50% reduction in bloodflow to the skin over ribs in steers occurred at an ergovaline concentration 590 µg ergovaline kg^{-1} DM at an ambient temperature of 32 °C (Rhodes et al., 1991). Elevated core body temperature in yearling beef bulls occurred after consumption of 40 µg ergotamine kg^{-1} BW (Schuenemann et al., 2005). Elevated rectal temperature as measured in yearling rams fed 33.7 µg ergovaline kg^{-1} BW when ambient temperature was above 22°C (Burke et al., 2006).

Fat necrosis is another physical sign of fescue toxicosis. Fat necrosis is condition arises from an alteration in lipid metabolism (Smith et al., 2004). It can be seen as the

mineralization of adipose tissue with calcium salts (Vernon, 1981). Animals can die as stone-like abdominal fat displaces or crushes internal organs (Smith et al., 2004; Wolfe et al., 1998).

As mentioned above in the economics section, a clinical sign of fescue toxicosis is reduced fertility (Looper et al., 2009, Burke et al., 2006, Gazvani et al., 2000). Females may fail to breed or fail to carry a calf to term because of obstruction caused by fat necrosis (Wolfe et al., 1998) or poor quality sperm (Burke et al., 2006).

Serum prolactin is consistently suppressed in cattle fed toxic tall fescue (Aiken et al., 1998, Lipham et al., 1989, Hurley et al., 1981). Prolactin is a peptide hormone that plays a role in lactation and in immune response. Prolactin production is suppressed by an increase in dopamine concentration. Serum prolactin suppression in ergot-exposed cattle indicates an alteration in dopaminergic activity in the anterior pituitary gland (Oliver, 2005). Prolactin concentration in crossbred heifers fed a feed concentrate containing endophyte-infected, tall fescue seed was less than their baseline concentration and crossbred heifers fed ergovaline-free feed concentrate 27 h after initial exposure (Aiken et al., 2009).

Prolactin concentrations may have a positive relationship with ambient air temperatures (Tucker et al., 1991). Prolactin suppression may hinder hair loss in spring and summer leaving the animal with a long coat during the warmest time of the year (Oliver, 1997). Prolactin-suppressed animals have sought to cool themselves by standing in shade or standing water instead of grazing (Strickland et al., 1993) and therefore have expressed a change in grazing behavior.

Animal Behavior or Clinical Symptoms

Clinical symptoms or changes in animal behavior due to fescue toxicosis have been observed when ergovaline concentration is greater than 200 µg kg^{-1} DM in Missouri (Cornell et al., 1990), 390 µg kg^{-1} DM in Kentucky (Aiken et al., 2009) and 475 µg kg^{-1} DM in Oregon (Aldrich-Markham et al., 2007). A key behavior change is reduced forage intake (Aiken et al., 2009; Nihsen et al., 2004, Thompson et al., 1987). For example, reduced forage intake occurred in steers in Kentucky when the animals consumed 790 µg ergovaline kg^{-1} DM and ambient air temperature was 7.6 ± 2.9 °C (Aiken et al., 2009).

Tall Fescue

Tall fescue is thought to have arrived unintentionally in North America from Europe in the late 19th century. Development of tall fescue varieties was initiated in Oregon in 1907 and in Kentucky in 1931 (review by Browning, 2003). Tall fescue proved early on to be a persistent and grazing-tolerant forage grass; it was widely distributed as 'Kentucky-31', beginning in the 1940s (review by Browning, 2003; review by Stuedemann and Hoveland, 1988). Unknown at the time of its release was that 'Kentucky-31' tall fescue contained an endophytic fungus (Bacon et al., 1977) that produced ergot alkaloid compounds (Yates et al., 1985, Porter et al., 1981). The first modern documentation of health problems and poor production performance for animals grazing tall fescue was published in the late 1940s and early 1950s (review by Bacon, 1995, Stearns, 1953, Goodman, 1952, Cunningham, 1948).

Endophyte-infected tall fescue, such as 'Kentucky 31', can withstand drought (Hahn et al., 2008, review by Malinowski and Belesky, 2000, Hill et al., 1996, Elmi and

West, 1995) and pests (Bacetty et al., 2009, Timper et al., 2005, Lehtonen et al., 2005, Elmi et al., 2000, Kimmons et al., 1990, Johnson et al., 1985) because of its association with *N. coenophialum*. 'Kentucky-31' tall fescue is relatively cheap to establish and manage (Stuedemann and Hoveland, 1988).

Neotyphodium coenophialum

The presence of the fungus, now known as *N. coenophialum*, was proposed by Niell (1941) and verified by Bacon et al. (1977). The endophyte is an asexual anamorph of the *Epichloe* genus. *N. coenophialum* is vertically transferred through infected seeds and by tall fescue tillers. It is found in the intracellular spaces of tall fescue. *N. coenophialum* is concentrated at growing points, or nutrient sinks within the plant, such as the stem apical meristem and vertically within blade sheaths. The endophyte is non-branching with exceptions occurring in seedheads.

Ergot Alkaloids

Ergot alkaloids have been identified as a cause of fescue toxicosis. They have a basic structure in which the lysergic acid moiety establishes the skeleton and determines the alkaloid's characteristics (Böhm, 1985). Ergot alkaloids contain at least one N atom originating from an amino acid, and they have limited distribution within the plant host (review by Waterman and Harbone, 1998). They are indole derivatives substituted at the 3, 4 position. In ergot alkaloids, the C-8 position is substituted with a single carbon unit which may serve as the attachment site for side chains depending on the ergot alkaloid oxidation state (Keller et al., 1988).

Ergot alkaloids are a suite of secondary-metabolite mycotoxins based on *N*-heterocyclic compounds or on a tetracyclic ergoline ring system (Tudzynski et al., 2001; Mothes and Luckner, 1985). They are composed of half clavine alkaloid and half lysergic acid derivatives (Lyons et al., 1986; Gröger, 1985). Ergot alkaloids include ergolene acids, lysergic acid and lysergic acid amide derivatives, clavines and ergopeptine alkaloid (Garner et al., 1993).

Until the mid-1600s *Claviceps purpurea* [(Fr.) Tul.] and other *Claviceps* species growing on cereal rye (*Secale cereal* L.) and other grains were not identified as fungus but considered a deformed grain (review by Barger, 1931). *Claviceps* was considered the documented source of ergotism symptoms in humans as well as livestock until the last quarter of the 20th century. In Britain in 1858 and New Zealand in 1894, *Claviceps* infection and resulting ergot poisoning of rye and fescue pastures was blamed for the loss of livestock and income by some cattlemen and veterinarians (Charlton, 1894; review by Barger, 1931). *Claviceps* was noted by Neill (1941) to not be the sole potential source of ergot poisoning in tall fescue and ryegrass forage. Alongside and in absence of *Claviceps*, Neill (1941) identified an endophyte – a fungus living between plant cells – that grew within tall fescue and ryegrass and reproduced asexually.

In the visible absence of *Claviceps* sclerotia or ergot bodies, Maag and Tobiska (1956) identified a Colorado tall fescue pasture producing symptoms of ergot poisoning in cattle. An endophytic fungus, currently called *Neotyphodium coenophialum*, was identified in tall fescue by Bacon et al. (1977). Soon after the work by Bacon et al., ergot

alkaloid production was linked with *N. coenophialum* (Porter et al., 1981; Porter et al., 1979) in addition to *Claviceps*.

Ergovaline

Ergovaline is a prominent ergopeptine compound associated with *N. coenophialum* and fescue toxicosis (Hill, 2005; Schardl and Panaccione, 2005; Yates et al., 1985). Ergovaline accounts for over 84% of the ergopeptine alkaloid content (Lyons et al., 1986) and over 80% of identified ergopeptine alkaloids (Belesky et al., 1988). Ergovaline, like other ergopeptine alkaloids, can easily break down or undergo additional reactions (Garner et al., 1993) and experience rapid turnover (Neumann, 1985). Compartmentalized, ergovaline storage in vacuoles and lipid droplets, plus rapid turnover of alkaloids, protects the fungus from its own toxic compounds (Neumann, 1985). Other ergopeptines including ergosine, ergosinine and ergotamine, are also associated with clinical signs of fescue toxicosis (Porter et al., 1981).

Plant Nutrients

Manipulating N nutrition of plants to limit fescue toxicosis was one of the first alkaloid management strategies attempted (Malinowski and Belesky, 2000). Nitrogen management may yet provide a way to limit fescue toxicosis in cattle. With increasing N fertilization, ergot alkaloid concentration can increase in both field and controlled-environmental experiments (Rottinghaus et al., 1991, Belesky et al., 1988, Lyons et al., 1986).

The form of N has been reported in the literature to be as important to ergot alkaloid production as N application rate. Nitrogen in soil is found in organic, inorganic

and gas forms. Organic N often held as soil organic matter can constitute up to 90% of soil N (Olk, 2008). Organic N must be mineralized to inorganic N before it is available for plant use (Beegle et al., 2008). Inorganic N in the form of nitrate (NO_3^-) can be an indicator of plant available N, as it is often in the greatest quantities of any plant available N form. Exchangeable ammonium (NH_4^+) is an important plant available N form found at levels less than NO_3^- because of rapid nitrification of NH_4^+ to NO_3^- (review by Bronson, 2008). Inexchangable NH_4^+ and exchangeable NH_4^+ together in the soil can have a NH_4^+ to NO_3^- ratio of 1,000:1 (review by Miller et al., 2009). Inorganic N, particularly NO_3^-, moves in precipitation surface runoff where it enters the surrounding aquatic environments. Excess inorganic N can cause havoc in naturally nutrient poor systems leading to algae blooms, fish kills and degradation of potable water supplies. Nitrogen gas as nitrous oxide (N_2O) and other nitrogen oxides are released into the atmosphere during nitrification and denitrification (review by Bronson, 2008; review by Marschner, 1995).

Fungi that use nitrates can also use ammonia as an N source, but not all fungi using ammonia can use NO_3^-. Fungi unable to thrive with only ammonia as an N source are likely to be parasites that developed specialized N nutrition (Jennings, 1995). Ammonium (Naffa et al., 1998; Kulkarni and Nielsen, 1986) but not NO_3^- (Kulkarni and Nielson, 1986) has been shown to support *in situ* growth of *N. coenophialum*. According to Arechavaleta et al. (1992), high rates of ammonium fertilizer in the field have a greater impact on increasing ergot alkaloid concentration in tall fescue than NO_3^- fertilizer. Both ammonium and glutamine, the first amino acid formed from NH_4^+, can prevent the

synthesis of other N source membrane-uptake proteins and, possibly more importantly, nitrate-utilization enzyme synthesis (Deacon, 2006; review by Pateman and Kinghorn, 1976).

Urea is neither an amino acid nor an inorganic molecule. Urea conversion to 2 molecules of ammonia and a single molecule of CO_2 requires the enzyme urease (Pateman and Kinghorn, 1976). Urea conversion takes place after direct entry into roots or in the soil prior to root uptake (review by Marschner, 1995). Kulkarni and Nielson (1986) reported urea did not support endophyte growth but Naffa et al. (1998) found that urea supported *Neotyphodium* spp. growth.

A variety of nutrients besides N are required for fungal growth. In a culture environment and a wide variety of carbon sources, *Neotyphodium* grows slowly. Free glucose converted into mannitol can supply the endophyte with energy (Naffa et al., 1998; Kulkarni and Nielsen, 1986). But glucose concentrations greater than 1% can reduce growth (Naffa et al., 1998). When the only carbon sources are pectin, cellulose, galaturonic acid or polygalacturonic acid, no fungal growth occurs; evidently *Neotyphodium* ssp. cannot utilize cell wall components as an energy source (Naffa et al., 1998; Kulkarni and Nielsen, 1986). *Neotyphodium* grew best with a variety of amino acids such as tryptophan, methionine, asparagines and glutamine (Naffa et al., 1998; Kulkarni and Nielsen, 1986).

As explained above, the form in which fertilizer nutrients are delivered to tall fescue roots, and eventually *Neotyphodium* spp., can influence ergot alkaloid production. Because of the ease of access to locally-produced poultry litter in the tall fescue region, N

from poultry litter used as a fertilizer plays a large role in tall fescue pasture productivity. Nitrogen form and content in poultry litter depends on the poultry feeding program, the environmental conditions, the poultry production level, and the storage and applications methods for the litter (review by Beegle et al., 2008).

Nitrogen in poultry litter is made up of urea, NO_3^- and NH_4^+. Nitrogen in poultry litter can be highly variable, even among samples from the same barn. When sampling poultry litter, N represents the *average* N content of a unit of poultry litter. Nitrogen content of poultry litter may not fully meet the immediate needs of the crop to be fertilized. It is accessed through two different "pools." One pool is "fast" and quickly mineralized, while the "slow" N pool was reported to be not as variable among poultry litter samples as the "fast" pool (Gordillo and Cabrera, 1997). Nitrogen that is immediately plant available can be viewed as an opportunity for the best use of poultry litter as a fertilizer (review by Beegle et al., 2008).

Urea or uric acid can potentially compose 40 to 60% of the N in fresh animal manure. Urea is readily available for mineralization. Urea is quickly mineralized in the presence of urease into inorganic NH_4^+ (review by Beegle et al., 2008). Uric acid concentration and the poultry litter's C:N ratio can be used to predict total mineralizable N (Gordillo and Carera, 1997). Most urea-N is mineralized in the first week of composting (Kessel et al., 2000). Urea in the barn can undergo hydrolysis to form $(NH_4)_2CO_3$, which raises the barn's poultry litter pH and triggers further conversion of NH_4^+ to NH_3 (review by Beegle et al., 2008).

There is little NO_3^- in litter and other manures (review by Beegle et al., 2008). Nitrate availability in a poultry litter-soil environment depends on soil temperature and soil moisture. Nitrate in warm, aerobic conditions becomes available as its precursor NH_4^+ undergoes nitrification. This nitrate moves with soil moisture mass flow and overland surface flow and undergoes denitrification (review by Beegle et al., 2008).

Ammonium in the poultry litter-soil environment mineralizes to NO_3^-. Of the NH_4^+ and other N compounds in poultry litter in the "fast" mineralization pool, 70 to 96% is mineralized during the first 24 h of incubation (Gordillo and Cabrera, 1997). While NH_4^+ can enter the plant, it is mineralized to NO_3^- or is stored in root cell vacuoles.

Ammonium can also convert to NH_3 gas if it is mineralized at the poultry litter surface or at the soil surface (review by Beegle et al., 2008). Ammonium concentration in the soil decreases as NO_3^- concentration increases; the ratio over time can be used to gauge rate of mineralization occurring in a soil or other medium (Sistani et al., 2008).

The amount of N in the soil available to the plant is only part of the story. Nitrogen amount required for a plant's optimal growth can be between 2 and 5% plant DW (review by Marschner, 1995). Nitrogen supply effects leaf growth and concentration of various plant constituents. It is required, for example, in the storage of lipids and in phytohormone production. Nitrogen is a vital component of chlorophyll *a*. Four N atoms link with magnesium in the center of the chlorophyll structure and the surrounding carbon structures. Nitrogen supply in green leaves is related to the galactolipid content that acts as structural components to chloroplasts. Nitrogen deficiency can limit production of phytohormones, or chemical messengers. Nitrogen is a structural

component of the phytohormone auxin; a limited nitrogen supply could hinder auxin signaling for plant cell division and activation and induction of enzymes (review by Marschner, 1995). With all of its roles, its primary function in plants may be to provide the N component for amino groups in amino acids (review by Maasthuis, 2009).

Nitrogen uptake into plants depends on soil conditions (review by Maasthuis, 2009). Nitrogen uptake by the plant root from soil solution requires high attraction for the nutrient, since N is present in low concentrations in the soil and individual root cells compete for the nutrient resources (review by Miller et al., 2009). Nitrogen uptake by plant roots appears possibly to have four different transport systems (review by Lea and Azevedo, 2006). Its electrical charge and direction of transport determine its membrane transport (review by Miller et al., 2009). In order to enter a plant, N in the form of NO_3^- requires a co-transport with at least two protons in order to overcome the energy barrier of the negative membrane potential of the root cell (review by Miller et al., 2009).

Once N enters a plant, it must be transported to where it is needed. As stated earlier, ammonium (NH_4^+) and nitrate (NO_3^-) are the primary N sources consumed by plant roots (review by Marschner, 1995). Ammonium transport rarely happens within a plant. The majority of NH_4^+ is converted in the roots into an organic N form. Ammonium and its equilibrium partner, ammonia (NH_3), are toxic to plants at low concentrations. (Beusichem et al., 1988). Nitrogen-rich products such as amino acids and amides move through the xylem to nutrient sinks in other parts of the plant (review by Marschner, 1995). Ammonium can be stored in root cell vacuoles, where low pH prevents the formation of NH_3. Ammonium conversion into nontoxic, N-rich compounds ideally takes

place in roots because leaves and stems cannot readily dispose of the byproduct protons (review by Marschner, 1995).

Nitrate is not found in phloem, but is is found in xylem as $K•NO_3$. Nitrate can be stored in the vacuoles of storage organs, shoots and roots. Nitrate must be reduced to NH_3 in order to be accessible for conversion into organic structures (review by Marschner, 1995). Nitrate reduction to NH_3 is moderated by two enzymes: NO_3^- reductase in the cytoplasm and nitrite (NO_2^-) reductase in the chloroplast. Nitrogen is passed via NH_3 into the formation processes of amides, amino acids, peptides and other compounds with N components. These N-rich, low-molecular weight organic compounds are further processed into, for example, proteins, coenzymes, membrane constituents and nucleic acids which are necessary for the plant's life processes (review by Marschner, 1995).

Phosphorus

Phosphorus is an essential macronutrient for plant, fungus and animal health. After N, P is the second most important element in forage production. For optimal vegetative growth, P concentration should occur in the range of 0.2 – 0.5% DM. Phosphorus is necessary for phospholipid production and nucleic acid formation and in cells' metabolic machinery as phosphate esters and pyrophosphate. It is the essential component of plant 'energy currency' adenosine triphosphate (ATP) (Brady and Weil, 2000; Schachtman et al., 1998; review by Marschner, 1995). The phosphorus used by plants originates from the soil environment in which the plant grows.

Fungus, such as the endophyte *Neotyphodium*, is dependent on the ability of its host plant to obtain enough P from the soil environment to meet the needs of both it and

the host plant. Fungal P requirements are similar to those of plants. Fungi in general have multiple ways of acquiring P. Phosphorus uptake can occur as a system-wide activity. The fungi can release phosphatase enzymes that cleave phosphate from organic sources in the surrounding environment. Fungi can lower external pH by releasing organic acids into the apoplast. Fungi can also physically seek, through branching, fresh nutrient zones of their environment (Deacon, 2006).

Neotyphodium species evolved in nutrient poor, intracellular spaces of grass and grass seed. In this sort of environment, fungal P uptake is determined by cellular pH. Should the intracellular environment pH and the endophyte's internal pH not be condusive to P transport across the fungal exterior, *Neotyphodium* cannot maximize its growth potential. Phosphorus can be stored in fungal vacuoles as orthophosphates along with secondary metabolites such as ergot alkaloid (Jennings, 1995). The endophyte was reported by Azevedo (1993) to be a P sink. Endophyte hyphae accumulated inorganic P thus acting as a reserve in tall fescue grown in low soil P conditions.

Production of secondary metabolites, such as ergot alkaloids, requires energy originating from ATP and NADPH. Ergot alkaloid synthesis is accomplished in two separate parts: synthesis of the peptide portion and synthesis of the lysergic acid moiety (Rutschmann and Stadler, 1978). Ergot alkaloids and ergovaline are derivatives of products of the shikimate-chorismate pathway. The shikimate-chorismate biosynthetic pathway begins with phosphoenolpyruvate and erythrose-4-phosphate and ends with tryptophan. Synthesis of ergovaline and other ergot alkaloids requires the prenylation of tryptophan. Then, decarboxylation, cyclization and oxidation produce lysergic acid

amide. Ergovaline forms by the condensation of the lysergic acid moiety with three amino acids: alanine, valine and proline (Schardl and Panaccione, 2005; Robbers, 1984)

Phosphorus fertilization has been reported to enhance ergopeptine alkaloid production in endophyte-tall fescue associations. A greenhouse experiment conducted by Malinowski et al. (1998) demonstrated how particular genotypes responded to P fertilization in different soil P environments. In this study, low-alkaloid-producing genotypes responded to increasing soil P by producing higher alkaloid concentrations as soil P increased. In contrast, the high-alkaloid-producing tall fescue genotypes produced 50% more ergot alkaloid when grown in the middle P treatment, which was 100 kg soil P ha^{-1}, than in the high P soil treatment, which was 200 kg soil P ha^{-1}. Production of ergovaline was reported to be a function of increased P availability and not associated with a specific endophyte-tall fescue genotype association (Azevedo, 1993). Their experiment did not report the influence of P fertilization on ergopeptine alkaloid production in wild-type *N. coenophilum* infected "Kentucky 31" tall fescue, which remains is unknown.

While P concentration may be high in a soil, very little of the element may be accessible for plant use. Most P in soil occurs in insoluble organic and mineralized forms that are unavailable to plant roots. Of the soil P that is soluble, most occurs in low concentrations and moves through the soil by diffusion. Phosphorus diffusion through soil spaces can be a slow process, taking 10^{-12} to 10^{-15} m^2 s^{-1} (Holford, 1997); this low concentration and the limited mobility render soluble P almost inaccessible by the plant.

Inorganic P (Pi) is the soluble form of phosphorus most readily available for plant uptake. The monovalent form of Pi, $H_2PO_4^-$, has the highest rate of uptake between pH 5.0 and 6.0. Plant availability of phosphate compounds is highest when the soil pH range is 6.0 to 7.0 (Brady and Weil, 2000). Inorganic P can be modified from organic P to Pi and mobilized by altering the soil solution pH, altering soil particle surfaces, anions competing for sorption sites with phosphate ions and anions chelating cations bound to P (review by Richardson et al., 2009). Phosphorus use during times of high rates of plant growth can strip the soil surrounding the plant roots, thus limiting plant and fungi growth.

Endophyte infection may or may not impact P uptake, but it may improve P use efficiency (Ren et al., 2007). In perennial ryegrass (*Lolium perenne* L.), the endophyte did not affect P uptake in a greenhouse, pot study. However, endophyte-infected perennial ryegrass had higher P concentrations in blade sheaths and blades than endophyte-free ryegrass, as long as P supply was adequate. The reverse was found when P was deficient (Ren et al., 2007).

In another greenhouse pot study (Rahman and Saiga, 2007), endophyte-infected tall fescue produced more aboveground biomass when grown in low P soil compared to a high P soil. Two endophyte-infected cultivars produced higher concentrations of P and manganese (Mn), regardless of soil type. The same endophyte species in a third tall fescue cultivar showed no difference in P concentrations between high and low P soils (Rahman and Saiga, 2007). Although endophytes do not control mineral concentrations in plants, the genetic potential of the tall fescue does controls mineral concentrations of its biomass.

Likewise, in a greenhouse pot study involving four tall fescue cloned genotypes, endophyte-infected tall fescue contained significantly greater concentrations of P, magnesium (Mg) and calcium (Ca) in shoots when the plants were grown at low soil P level (50 kg P ha^{-1}) than uninfected tall fescue (Malinowski et al., 1998). As P availability increased, shoot DM in the four endophyte-infected tall fescue genotypes was not greater than in uninfected tall fescue. Endophyte infection provided no benefit to tall fescue as soil P increased (Malinowski and Belesky, 2000).

The growth of mycorrhiza is reported by Omancini et al. (2006) to be hindered by the presence of *N. coenophialum* in tall fescue; this could further limit phosphorus uptake by tall fescue. Mycorrhizal associations increase surrounding plants' effective root length and root surface area, expanding the volume of soil available for nutrient exploration (Richardson et al., 2009). It is energetically more efficient for the plant to take in P from the mycorrhizal fungi than produce new roots to explore the surrounding rhizosphere (Deacon, 2006). Growth of mycorrhizal fungi has been reported to be obstructed by root exudates from *N. coenophialum*-infected tall fescue.

Phosphorous uptake by plants depends on rate of diffusion and the ability of root systems to intercept new P sources (Barber, 1984). Phosphorus uptake through an intermediary such as mycorrihizal fungi may play an important role in maintaining P concentrations within some plants (Brady and Weil, 2000).

Phosphorus uptake can be regulated by genes, proteins and enzymes (review by Antmann and Blatt, 2009). Lack of P can signal two types of genes: non-specific stress related 'early' genes responding within 24 h and the majority 'late' gene expression

responding to P deprivation occurring over a period of days. The ‘late’ genes affect the internal P metabolism and the architecture and function of roots (review by Richardson et al., 2009). Specialized transporters at the root-rhizosphere interface move inorganic P from soil solution (<10 µM) into the high P concentration (up to 1,000 µM) area found in the cytosol (Schachtman et al., 1998). Soon after entry into plant roots, Pi is converted to organic P; then is converted back to Pi before it is released into the xylem (review by Marschner, 1995).

Once in the xylem, Pi is transported to leaves and fruits. Inorganic P is stored in vacuoles if not utilized for metabolic functioning. Unlike N, Pi is not reduced and transported back to the roots for cycling within the plant. Excess Pi that might hinder metabolic processes is placed in vacuoles for later use during Pi deficiency or in fruit ripening. Cytosolic Pi content is tightly regulated, with vacuole Pi storage acting as a buffer. The vacuole Pi buffer insures metabolic stability for essential photosynthetic and reproductive processes, as nutrient availability can vary with weather conditions and diurnal cycles (review by Marschner, 1995).

Limestone

Limestone may impact fescue toxicosis by influencing plant growth and nutrient access in tall fescue, which could affect alkaloid production. Limestone is used as an agricultural soil conditioner to reduce levels of hydrogen (H^+) and soluble aluminum (Al^{3+}) in the soil. Small concentrations of Al^{3+} can be more detrimental to plant growth than larger concentrations of H^+ (review by Barber, 1984). Limestone maximizes crop yields when a soil contains an adequate number of pockets of neutralized soil to meet a

crop's nutrient demands, balanced against the crop's tolerance of toxic ions (review by McLean and Brown, 1984). The amount of limestone required to neutralize a soil and to meet a crop's needs varies from crop to crop. Limestone reaction rates are determined by limestone fineness, chemical components and physical composition.

Limestone ($CaCO_3$ or R-$CaCO_3$) is a salt acting as a base in acid soils (review by McLean and Brown, 1984). Limestone counteracts soil acidity caused by, for example, rainfall exceeding evapotranspiration, a soil's parent material, or by increasingly high N and sulfur (S) fertilizer input (review by Bolan and Hedley, 2003).

Calcitic limestone ($CaCO_3$) and dolomitic limestone [$CaMg(CO_3)_2$] are both common forms of limestone. Limestone effectiveness is measured by its $CaCO_3$ equivalence. Dolomitic limestone provides a higher neutralizing capacity than calcitic limestone due to Mg's lower atomic weight (review by Barber, 1984).

Limestone fineness can determine how fast cations are released into the soil environment. Limestone particles should be of a size that allows the lime to react completely with the soil within 2 to 3 y after application (review by Barber, 1984).

Limestone application in forage production serves multiple purposes: increasing base saturation to a near-neutral pH level, decreasing toxic soil elements to a level optimizing crop yields (review by McLean and Brown, 1984), and increasing cation concentration in forage to alleviate potentially fatal livestock disorders. Limestone use reduces a combination of soil factors – calcium (Ca) and Mg deficiency, Mn toxicity and Al toxicity -- that limit forage growth (review by McLean and Brown, 1984) and livestock production.

Limestone application can alter the micro- and macronutrient composition of forage plants. Changes in soil acidity affect plant accessibility to individual elemental nutrients. One study reported that with limestone treatments of 0, 1, 2, 4 and 8 t ha^{-1}, there was a negative correlation between the bromine (Br), cobalt (Co), chromium (Cr), Mn and zinc (Zn) composition of forage plants and the amount of limestone applied (Armelin et al., 2005). Magnesium expressed a positive correlation with the composition of forage plants, increasing with increasing limestone application rate, possibly because limestone increased soil pH.

Other soil amendments in addition to limestone can also influence soil and forage nutrient concentrations. In West Virginia, on an abandoned pasture unmanaged for 40 years, gypsum ($CaSO_4 \bullet 2H_2O$) applied at five different rates with dolomitic limestone or with a different Mg source, MgO or MgH, (nine treatments total) and at three fertilizer rates, altered soil and forage Mg, Ca, S, P and K concentrations (Richey and Snuffer, 2002). Before the study, the pasture contained red fescue (*Festuca ruba* L.), poverty grass (*Danthonia spicata* L.) and broom sedge (*Andropogon virginicus* L.) representing 28% of the pasture's botanical composition. Broadleaf plants comprised 66% of the original plant population. 'Canvy' Kentucky bluegrass (*Poa prantensis* L.), orchardgrass, and 'Kentucky-31' tall fescue were no-till seeded in July 1994 and March 1995. Forage and soil samples were collected from 1994 to 1997. Soil pH_s increased from an initial 4.3 to over 4.5 with increasing total calcium carbonate equivalency (TCE). Use of gypsum alone decreased soil Mg concentration and pH_s. When gypsum was used alongside dolomitic limestone, the soil maintained adequate levels of Mg to meet plant needs. Plant

Mg concentrations reflected changes in soil Mg. The researchers reported no relationship between plant P and K concentrations and treatment TCE. They explained this was because reduced Al^{3+} activity allowed plants to have access to additional soil P and K through rapid root growth, mychorrhizae development and finer root branching. Forage yield increased with application of limestone, decreasing soil pH and improving plant uptake of P and K. Tall fescue was more sensitive to lower soil acidity than orchardgrass (Richey and Snuffer, 2002).

In a study in Nova Scotia, dolomitic limestone altered soil pH in planted mixed forage pasture (Gupta et al., 1971). The field had been unmanaged for 10 years. Five rates of dolomitic limestone, incoluding 0, 1.0, 2.4, 4.4 and 8.1 t ha^{-1}, was incorporated into the soil. A forage mixture of 'Scotian' oats (*Avena sativa* L.), alfalfa, (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), alsike clover (*Trifolium hybridum* L.), timothy (*Phleum pratense* L.), bromegrass (*Brome inermis* Leyss.) and orchardgrass was planted. Four rates of P-K fertilizer were applied in spring. One rate of ammonium nitrate was applied in spring after first cutting. Molybdenum (Mo), copper (Cu), boron (B), Mn and Zn were sampled in the mixed forage tissue and soil. There was no interaction between lime and fertilizer on micronutrient content. Plant tissue Mo increased with increasing soil pH up to pH 6.6. Zinc, Mn, B decreased with increasing soil pH. Plant tissue Cu increased up to pH 5.6 but no further increase was measured above pH 5.6. Only Mo was affected by increasing fertilizer rates; Mo decreased with increasing P-K fertilizer rates (Gupta et al., 1971).

Herbicide

The search for a technique to limit fescue toxicosis in cattle has included experiments with herbicides. Prior to the first half of the 1990s, when the methodologies to measure ergovaline (Rottinghaus, et al., 1991) and ergot alkaloids (Hill and Agee, 1994) directly were published, the toxin load of tall fescue was indirectly measured by livestock production. Such indirect measurements were seen in studies that explored the use of plant growth suppressing herbicides. In these studies, chemical tools in some way reduced fescue toxicosis symptoms, as observed through forage quality and cattle performance; much of the reduction of fescue toxicosis symptoms was the result of the reduction in seedhead production.

Chemical tools that included herbicides such as mefluidide, which physiologically suppresses seedhead development, were explored by forage scientists in an effort to decrease the toxicity of tall fescue. These growth regulators can delay maturity and extend the peak quality of tall fescue forage beyond late spring. Chemical tools that limit seedhead development should reduce livestock exposure to toxins, because blades and blade sheaths contain at least 5 times less ergovaline concentration than seedheads (Rottinghaus et al., 1991). Turner et al. (1990a) reported spring-applied mefluidide increased forage intake and grazing animal performance by 20 to 30%. Mefluidide-treated tall fescue grazing animals had significantly improved seasonal average daily gain over aimals grazing untreated tall fescue (Allen et al., 1988; Allen et al., 1986; Garrett et al., 1986). Mefluidide delayed plant maturation, improving the quality and effective use

of tall fescue into August compared to untreated tall fescue with biomass production which peaked in June (Turner et al., 1990b).

Amidochlor is another growth regulating herbicide. Amidochlor could provide a method for limiting seedhead production. Herbicide effectiveness may be limited by other pasture management decisions including grazing intensity (Roberts and Moore, 1990) and environmental influences such as drought (Reynolds et al., 1993a).

Sethoxydim is a compound functionally similar to clethodim. It almost eliminated seedhead production after either a spring or winter application. Sethoxydim application may decrease overall forage yield, which would be unfavorable for hay production (Reynolds et al., 1993a) if biomass production were the primary management goal.

Past studies have found application of clethodim in autumn reduced seedhead numbers by almost half compared to the untreated control. Clethodim, when applied in March, almost eliminated seedhead production. Clethodim decreased forage yield and quality similarly to other cyclohexanedione compounds (sethoxydim and mefluidide) (Reynolds et al., 1993b). Herbicides such as clethodim could provide an important toxin reduction tool in wild-type endophyte infected tall fescue.

Clethodim is rapidly absorbed into a grass plant. In one study, two-thirds of the total applied ^{14}C-clethodim had been absorbed in Bermudagrass (*Cynodon dactylon* [(L.) Pers.]) blades within 24 h after application. Total applied ^{14}C-clethodim entering the plant leveled off 12 h after application (Nandula et al., 2007). Most ^{14}C-clethodim remained in the blade. The percentage of ^{14}C-clethodim that did move into the shoots fit a linear

equation from 0 to 72 h after application. The largest percentage of ^{14}C-clethodim in the shoot, 14% of total ^{14}C-clethodim applied, was from the ^{14}C-clethodim and crop oil concentrate plus ammonium sulfate treatment. A percentage of ^{14}C-clethodim, no greater than 8% of total ^{14}C-clethodim applied, moved into the roots (Nandula et al., 2007).

Clethodim, like other cyclohexanedione herbicides such as sethoxydim, inhibits chloroplast acetyl-coenzyme A carboxylase (ACCase) in *Gramineae* and *Geraniaceae* families (Rendina and Felts, 1988). There is a difference in response between chloroplast and cytosolic ACCase synthesis pathways, as well as a difference in response between monocotyledonous and dicotyledonous plants.

Two separate genes have been identified to encode individually for cytosolic and chloroplastic synthesis in *Gramineae*. Plastid ACCase is essential for primary fatty acid synthesis (Yu et al., 2007). Plastid ACCase represents more than 80% of total ACCase activity in cyclohexanedione-susceptible plants. In most plant species chloroplastic ACCase is a heteromeric enzyme, but in *Gramineae* species chloroplastic ACCase is homodimeric. (Yu et al., 2007; review by Délye et al., 2005). The structural difference between the homodimeric and heteromeric enzymes is utilized to develop herbicides for control of susceptible grass species in dicotyledonous field crops.

Malonyl-CoA, a three-carbon intermediate, is required for fatty acid synthesis. Malonyl-CoA's formation from acetyl-CoA requires ACCase in the two-step ACCase reaction. The ACCase reaction is the first committed step for fatty acid synthesis. First, a bicarbonate (HCO_3) carboxyl group is transferred to a biotin moiety through an ATP-dependent reaction. Then, the biotinyl group transfers CO_2 to acetyl-CoA with

transcarboxylase to yield malonyl-CoA (Burton et al., 1989; Rendina and Felts, 1988; Nelson and Cox, 2005). Without malonyl-CoA, fatty acid synthesis can continue using acetyl-CoA but at a much reduced rate (Burton et al., 1989). Cyclohexanedione-resistant grass may be able to compensate for loss of chloroplastic ACCase production of malonyl-CoA using cytolsolic ACCase (Yu et al., 2007). Without fatty acid synthesis, processes within the growing plant are limited, including the development of seedheads and culms, the primary locations for *Neotyphodium* growth and ergot alkaloid synthesis and storage.

Ergopeptine Fluctuation

As stated above, ergovaline concentrations measured in tall fescue depend on the growth and development of the tall fescue plant and *Neotyphodium*'s interactions with the host plant. Its concentrations are reported to fluctuate in tall fescue throughout the growing season in grazed and nongrazed conditions. In a Missouri grazing study (Peters et al., 1992), ergovaline decreased in cattle extrusa from 330 µg ergovaline kg^{-1} in June to 154 µg ergovaline kg^{-1} in August.

Fluctuation in ergovaline concentration during the growing season in a Missouri field study was reported by Rottinghaus et al. (1991). 'Kentucky-31' tall fescue samples were hand-clipped weekly from early May to early November in 1987 and 1988. The plants were divided into grass blades, blade sheath with culms and seedheads in May through June samples. Ergovaline concentration differed throughout season and among plant parts. In 1988, ergovaline concentration in stem with blade sheath decreased from a high of 1,083 µg ergovaline kg^{-1} DM in early May to 399 µg ergovaline kg^{-1} DM in late June. Composite sampling was initiated in early July. Ergovaline declined from 340 µg

ergovaline kg^{-1} DM in early July to 166 µg ergovaline kg^{-1} DM in early August in whole aboveground, composite samples after seedhead maturation. It peaked a second time during early October regrowth, with concentrations reaching 839 µg ergovaline kg^{-1} DM (Rottinghaus et al., 1991).

Under grazing conditions in Georgia, Belesky et al. (1988) measured ergovaline concentrations in 'Kentucky-31' tall fescue. Grazed tall fescue, fertilized at two N rates, was clipped at 3- to 4-cm stubble height at 4-wk intervals in the spring of 1983 and throughout the entire 228-d growing season of 1984. The study included the comparison of a high ergovaline-producing endophyte and a low ergovaline producing endophyte. For the high ergovaline producing endophyte in 1983 and both genotypes in 1984, ergovaline concentration across Georgia's spring and summer, peaked on calendar day 150 (late May), regardless of N fertilizer rate. Ergovaline concentration decreased after calendar day 178 (late June) in both endophyte genotypes and both years. After day 178, ergovaline in the 336 kg N ha^{-1} treatment increased to peak at calendar day 300 (late October). The high ergovaline-producing endophyte's 1984 ergovaline concentration exceeded 1,000 µg ergovaline kg^{-1} in late October; this concentration had more than tripled when compared to measurements in late May.

Greenhouse studies that measure ergovaline and total ergot alkaloid concentrations in tall fescue allow for control of soil and ambient conditions that are not feasible in field studies. In one greenhouse study, ergovaline concentrations in tall fescue clipped at the soil level decreased between month 2 after germination and month 4 after

germination. Ergovaline concentration particularly declined in plants clipped biweekly to a height of 5 cm (Salminen and Grewal, 2002).

Ergovaline concentration is understood to be higher in the blade sheath than the blade (Rottinghaus et al., 1991). However, results from Salminen and Grewal (2002) do not support the hypothesis that plants with a greater proportion of sheath compared to blade should have greater ergovaline concentrations. In their study, ergovaline concentration was reduced when a greater proportion of sheath was harvested. The researchers suggested this resulted from reallocation of carbon to the construction of new plant tissue and not from secondary metabolite production (Salminen and Grewal, 2002; Belesky and Hill, 1997).

In a study of established tall fescue cores brought from the field into a greenhouse (Salminen et al., 2003), ergovaline concentration produced a month x height interaction. Ergovaline concentration decreased from month 1 to month 2 when clipped weekly to heights of 5 and 7.5 cm. There was no change in ergovaline concentration over time when tall fescue was maintained at 2.5 cm. Ergovaline concentration in month 2 was lowest in tall fescue clipped weekly to a height of 5 cm; it was greatest in tall fescue clipped weekly to a height of 2.5 cm (Salminen et al., 2003). The 2.5-cm tall fescue was expected to have the highest ergovaline concentration because of the 2.5-cm plants' high sheath: blade ratio. The 7.5-cm tall fescue was expected to contain the lower ergovaline concentration, but this is not what was observed. Salminen et al. (2003) suggested that their data reflected an increase in ergovaline synthesis in clipped tall fescue and not change in a shoot-to-sheath ratio.

With a growth chamber, ergot alkaloid concentration can be monitored with even more control of environmental conditions than with a greenhouse. Ergot alkaloid concentration in tall fescue was monitored in a 6-week-long growth chamber study (Belesky and Hill, 1997) with two different endophyte genotypes-tall fescue associations clipped to a height of 5 and 10 cm. Leaf age and host-endophyte association produced significantly different ergot alkaloid concentrations. Ergot alkaloid concentration was greatest in the total blade less than 2 wk old in one genotype, and 2-4 wk old total blade material in the other genotype. Ergot alkaloid concentration was lowest in the total blade over 6 wk old, regardless of genotype (Belesky and Hill, 1997). They suggested ergot alkaloid concentration could be managed with defoliation and host-endophyte association.

Information provided by the above experiments may explain physiological processes, but greenhouse experiments do not replicate the challenges tall fescue and *Neotyphodium* encounter in a heavily grazed pasture. Greenhouses and growth chambers maintain a constant environment, which may not affect ergovaline or ergot alkaloid production and storage. Ergovaline concentration's summer decline was suggested by Belesky et al. (1988) to be due to photooxidation metabolites and high light intensities known to effect alkaloid structural integrity (Hellberg, 1957). Age of leaf blade or sheath may also affect ergovaline concentration. According to Tan et al. (2001), metabolic activity and ergovaline production of *Neotyphodium* mycelia in perennial ryegrass peaked *in planta* when an individual blade and blade sheath attained its maximum length of 20 cm in less than 20 days. A continuously clipped pasture should have no seedheads;

therefore, possibly reduced ergovaline concentrations. Accumulation of ergovaline and ergot alkaloid stored in *Neotyphodium* cell vacuoles and lipid droplets (Christensen et al. 2008) may not be detected in field studies with few sampling dates or in short term greenhouse experiments.

In a non-harvested pasture of tall fescue at the University of Missouri Forage Systems Research Center outside Linneus, Missouri, ergot alkaloid concentrations in endophyte-infected tall fescue declined by over 50% from December to February (Curtis and Kallenbach, 2007). The two year study included two 84-d grazing periods in early December through late February in 2004-2006. Ergot alkaloid concentrations were determined from pastures with three endophyte levels, 20, 51 and 89%. Tall fescue pastures were grazed to a height of 8-cm in August of each year, fertilized and allowed to accumulate until strip-grazing was initiated in early December. Researchers pooled ergot alkaloid concentration data among the two years. The high, endophyte-percentage pasture contained 919 µg ergovaline kg^{-1} DM in early December and 450 µg ergovaline kg^{-1} DM in late February. The middle, endophyte-rate treatment declined from 323 µg ergovaline kg^{-1} DM in early December to 168 µg ergovaline kg^{-1} DM in late February (Curtis and Kallenbach, 2007).

In a study conducted at the Southwest Missouri Research and Education Center near Mt. Vernon, three varieties of tall fescue, including endophyte-infected tall fescue, ‘Kentucky-31, ‘HiMag’ endophyte-free and ‘HiMag’ with nontoxic-endophyte were sampled for forage quality and ergovaline analysis (Kallenbach et al., 2003). Plots were clipped to a height of 8-cm in mid-August and fertilized with 56 kg N ha^{-1}. Four, 250-kg

steers per replicate grazed from mid-December to mid-March 1999-2001. Only ‘Kentucky-31’ produced ergovaline, as expected. In this cultivar, ergovaline concentration declined by 85% from mid-December to mid-March in both years. Ergovaline concentration decreased in the first year from mid-400s µg ergovaline kg^{-1} DM and in year two from upper-100 µg ergovaline kg^{-1} DM in mid-Dec to less than 100 µg ergovaline kg^{-1} DM in mid-March of both years. Brief warm periods in winter, Kallenbach et al. (2003) suggested, initiated small amounts of new growth; at the same time warm temperatures accelerated decay of older tissue.

In a two-year, 120-d grazing study in Missouri, ergovaline concentration decreased from June to August (Peters et al., 1992). Angus and Simmental x Angus cows with calves grazed ‘Kentucky-31’ tall fescue, endophyte-free ‘Moark’ tall fescue or ‘Hallmark’ orchardgrass mid-May to mid-September. Esophageal extrusa mean ergovaline concentration in ‘Kentucky-31’ tall fescue was 330 µg ergovaline kg^{-1} in June, twice that measured in August, 154 µg ergovaline kg^{-1}. Esophageal extrusa samples representing ergovaline concentration in spring growth were collected in June. Paddocks were mowed in late June to remove seedheads and culms. Ergovaline concentration in extrusa was, again, sampled in August. Ergovaline concentration measured in 1989 was suggested by the authors to be more representative of a cooler and wetter year when compared to hot and dry 1988 (Peters et al., 1992).

Summary

To summarize, field management practices have the potential to influence toxic ergovaline and ergot alkaloid concentration in tall fescue forage. Field management of N

and P, regardless of form, altered the toxin load of tall fescue. Field use of the herbicide clethodim reduced ergopeptine concentrations. Clipping of tall fescue forage also decreased toxin concentrations. More field based research is needed to help us understand the effects the form of N and P fertilization, limestone, and repeated clipping management on toxin concentrations. Field based research results also need to undergo an economic analysis. Field based research combined with economic analysis will identify which management practices reduce the toxin load in tall fescue and which are affordable.

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Chapter 2: The Effect of Clethodim Herbicide on Ergovaline and Ergot Alkaloid Concentrations

As discussed in Chapter 1, a low rate of clethodim herbicide (Fig. 2.1) applied in either spring or autumn may partially resolve cattle production problems associated with fescue toxicosis (Reynolds et al., 1993a, 1993b). Clethodim is a post-emergent herbicide that interferes with plastid fatty acid production and inhibits reproductive maturity in grasses. Its detrimental affect on seedhead development should reduce ergovaline (Fig. 2.2) and ergot alkaloid concentrations in toxic 'Kentucky 31' tall fescue, because seedheads contain up to 5 times more ergovaline than grass blade sheaths and blades (Rottinghaus et al., 1991). Clethodim is a chemical tool that could assist farmers in obtaining levels of cattle production that otherwise would be unrealized due to fescue toxicosis syndromes.

Objective

The objective of this study was to determine if a low rate clethodim application in autumn would affect ergovaline and ergot alkaloid concentration in toxic endophyte-infected 'Kentucky-31' tall fescue forage the following summer.

Methods and Procedures

Experimental Site

The study was conducted 2004 and 2006 on an established *N. coenophialum*-infected tall fescue 'Kentucky 31' grass stand (90% endophyte infection) located on the Southwest Missouri Research and Education Center near Mt. Vernon, Missouri (37°

04’N; 93° 53’W, elevation 351 m). The soil was a moderately well drained Hoberg silt loam (fine-loamy, siliceous, active, mesic Oxyaquic Fragiudalf). The site has a fragipan of dense clay at a depth of 40 to 92 cm and when unmanaged forms a perched water table 30 to 92 cm from the surface from December to March. The percentage of gravel size rock or larger below the fragipan layer can be as high as 70% of soil composition. This soil overlays karst structures representative of conditions found on the Springfield Plateau immediately west of the Ozark Mountains (Huges, 1982).

Twenty 7.6 m X 3.0 m plots were randomly assigned to be 10 treated or 10 non-treated control units. There was a 1.5 m alley between the four rows. The study was moved every year. Spring monthly high and low air temperature (°C) for 2005 and 2005 are presented in Fig. 2.3. Spring monthly precipitation sum for 2005 and 2006 and the thirty year average are presented in Fig. 2.4 and annual precipitation Fig. 2.5.

Application of 189 ml ha^{-1} “Select” brand clethodim with 2.5 L crop oil ha^{-1} occurred on 9 November 2004 and 7 November 2005. The plot was fertilized with 33.6 kg N ha^{-1} in mid-November each year.

Sample Collection and Laboratory Analysis

Forage was hand-harvested on 7 June 2005 and 7 June 2006, clipping 5 cm from soil surface before seed shatter. Samples weighing approximately 100 g wet weight were immediately placed in storage at -5°C. Samples were freeze-dried, ground through a 2-mm screen with a mill and then a 1-mm screen with a cyclone mill.

Ten grams of each sample was submitted for high pressure liquid chromatography (HPLC) analysis following the methods of Rottinghaus et al. (1991) for ergovaline

concentration. Between 2 and 3 g samples were scanned with near-infrared (NIR) spectroscopy following the procedures of Roberts et al. (1997, 2005) for total ergot alkaloid concentration. Instruments included a Pacific Scientific 5000 scanning monochromator (NIRSystems, Silver Spring, MD) with commercial software developed by Infrasoft International (Port Matilda, PA). The reference method for total ergot alkaloid concentration was Hill and Agee (1994) procedure using commercial ELISA test kit (Catalog no. ENDO899-96p; Agrinostics Ltd., Watkinsville, GA). The mean and standard error of calibration were 362 ± 56 µg total ergot alkaloid kg^{-1} DM; the standard error of cross-validation was 78 µg total ergot alkaloid kg^{-1} DM. The squared correlation coefficient for calibration was 0.96, and 1 minus the variance ratio was 0.89.

Statistical Analysis

Ergovaline and ergot alkaloid data were analyzed as a randomized, complete block with 2 treatments and 10 replicates per treatment per year. Year was considered as a random effect and clethodim treatment as a fixed effect. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

Results and Discussion

Ergovaline Concentration

There was a year x treatment interaction for ergovaline (Table 2.1, Fig. 2.6). Data below are discussed comparing year and clethodim treatment (Fig. 2.6) in order to explain the interaction.

The influence of year can be seen by the 33% greater ergovaline concentration in 2006 than in 2005. Ergovaline concentration in clethodim-treated tall fescue was less

ergovaline than control samples in both years (Fig. 2.6). The interaction occurred because the magnitude of the clethodim effect was inconsistent across years. In 2005 clethodim-treated forage contained 300 µg ergovaline kg^{-1} DM and control forage contained 492 µg ergovaline kg^{-1} DM, which is a difference of nearly 200 µg ergovaline kg^{-1} DM. In 2006 the difference between treated and control forage was nearly 700 µg ergovaline kg^{-1} DM, as clethodim-treated tall fescue forage contained 257 µg ergovaline kg^{-1} DM and control forage contained 941 µg ergovaline kg^{-1} DM (Fig. 2.6).

One cause of the difference in ergovaline between 2005 and 2006 was weather (Fig. 2.3, 2.4 and 2.5). Reduced precipitation is one of the primary factors that limits plant growth (Patton et al., 2007, Blonski et al., 2004). Wilhelm and Nelson (1978) reported that the tall fescue in their field study had greater leaf elongation, 8.54 mm d^{-1}, in autumn than compared to the summer, 4.15 mm d^{-1}. They attributed the decrease in summer leaf growth to higher temperatures and lower plant-water status. Leaf elongation in growth chambers slowed and recovered on a diurnal cycle as the growth chamber went from light to dark and decreased the temperature from 20°C to 15°C and then returned to light and 20°C conditions. They attributed this twice daily alteration in plants' response to its water status (Wilhelm and Nelson, 1978). It is important to realize that a limitation in plant growth can also be regarded as a limitation in microhabitat for the endophyte .

Consider the relationship between precipitation and ergovaline in this study. In March, April and May of 2005, precipitation was below the 30-y average (Fig. 2.5). In March of 2005, the year of low ergovaline concentration, precipitation was 66% of the 30-y average; in May of that same year, precipitation was only 30% of the 30-y average.

In contrast, precipitation in April and May of 2006 was greater than the monthly 30-y average.

Ergot Alkaloid Concentration

In this experiment with clethodim, the response of ergot alkaloid concentrations was similar to that of ergovaline. There was a year x treatment interaction (Table 2.1, Fig. 2.7), which prohibited pooling by treatment or year.

Even with the year x clethodim treatment interaction, tall fescue forage had greater total ergot alkaloid concentrations in 2006 than in 2005 (Fig. 2.7). The interaction occurred because the effect of clethodim differed in magnitude between 2005 and 2006. In 2005, untreated control plants contained 132 µg total ergot alkaloid kg^{-1}DM more than clethodim-treated tall fescue. In 2006, control contained 207 µg total ergot alkaloid kg^{-1} DM more than the clethodim-treated tall fescue.

As stated above, timely precipitation has a strong influence on plant growth (Patton et al., 2007; Blonski et al., 2004), and by association, extension of endophytic mycelia. Precipitation in the months prior to harvest in 2005 were less than the 30-y average. Precipitation in the months prior to the 2006 harvest were at or above the 30-y average. Without adequate moisture, plant and by association *Neotyphodium* hyphae growth is limited (Schmid et al., 2000).

In the case of ergot alkaloids, which include compounds produced by the surface fungus *Claviceps purpurea* [(Fr.) Tul.] infects *Lolium* species, it is likely that the wet weather of 2006 increased ergot alkaloids by creating conditions for surface infection;

Claviceps toxicity is maximized by a wet spring followed by a warm summer (review by Barger, 1931).

Conclusions

In this study, clethodim reduced ergovaline concentrations by as much as two-thirds in endophyte-infected tall fescue forage. Amount of precipitation received in the months prior to harvest at this location may have a significant impact on ergovaline concentrations independent of annual precipitation total. Further research into low-rate herbicides and chemical growth regulators, used at a paddock level, could help us better understand effects on reducing ergovaline in *N. coenophialum*-infected tall fescue and the effect on livestock production.

Figures and Tables

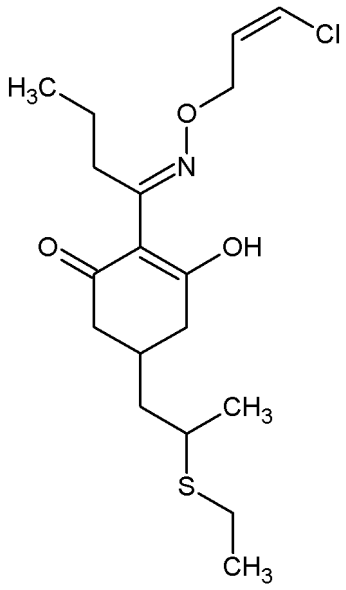

Figure 2.1. Structure of clethodim (redrawn from Redina and Felts, 1988).

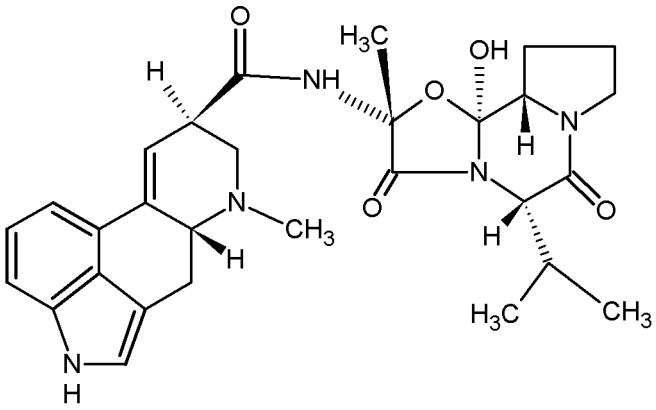

Figure 2.2. Structure of ergovaline (redrawn from Brunner et al., 1979).

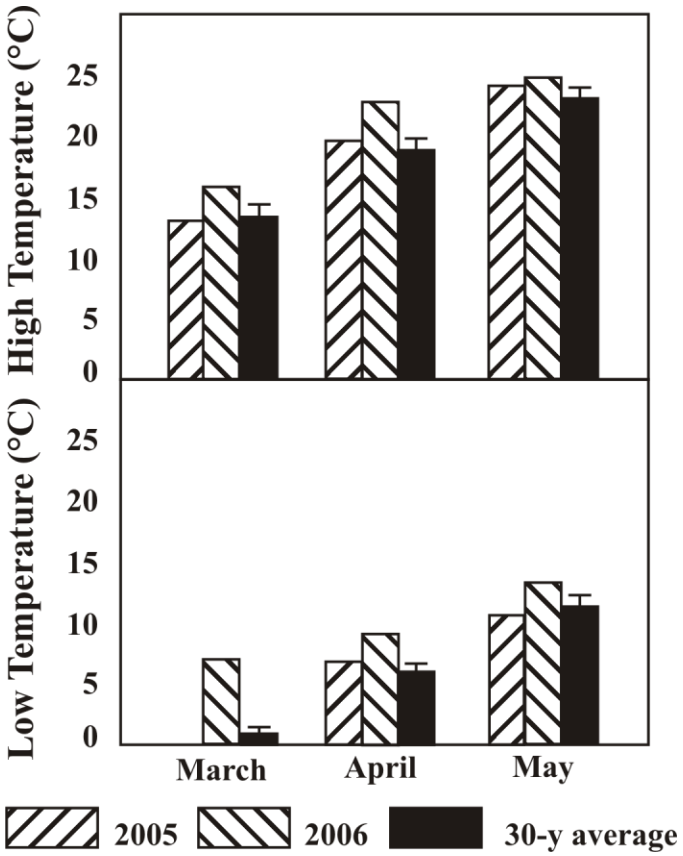

Figure 2.3. Average high and low air temperatures for March, April, May and the 30-y average (1977 to 2006) at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Average low air temperature for March, 2005 was 0.1 °C. Error bars represent 2 SE for the 30-y average.

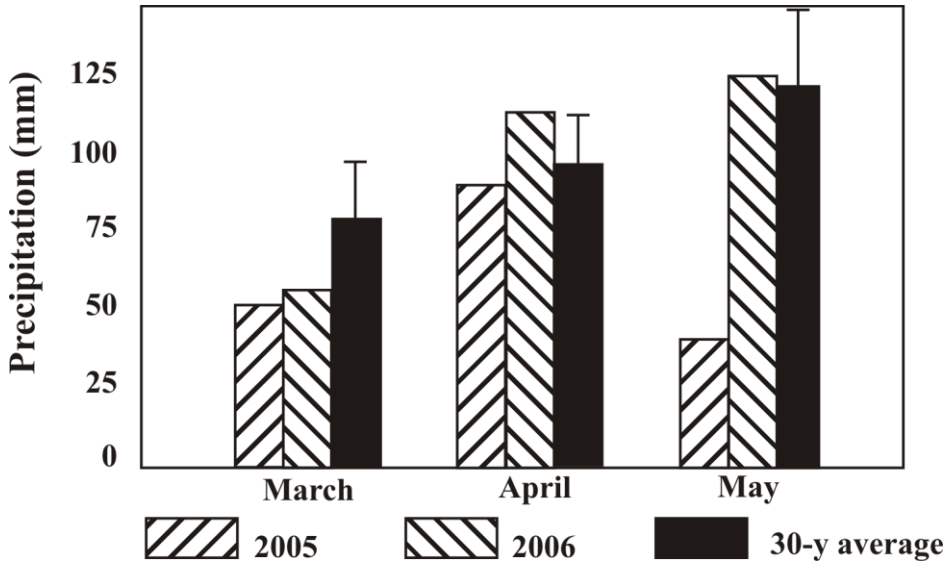

Figure 2.4. Total monthly precipitation for spring months prior to harvest for 2005, 2006, and the 30-y average (1977 to 2006) for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Error bars represent 2 SE for the 30-y average.

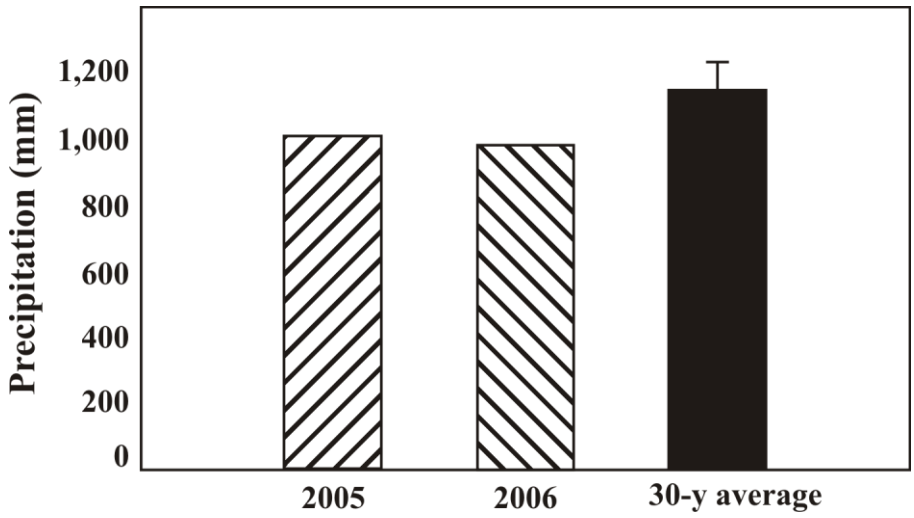

Figure 2.5. Annual precipitation for 2005, 2006 and the 30-y average (1977 to 2006) for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Error bars represent 2 SE for the 30-y average.

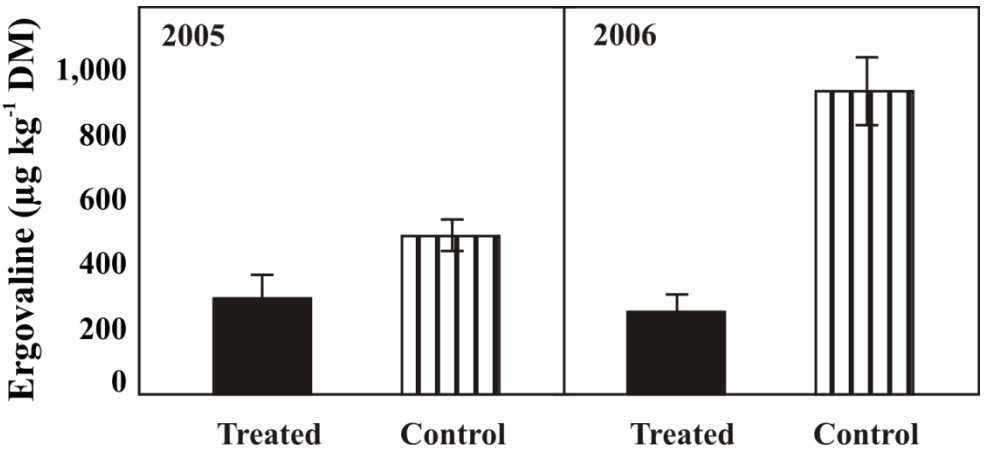

Figure 2.6. Ergovaline concentration between clethodim-treated and control tall fescue forage in 2005 and 2006 at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Error bars represent 2 SE.

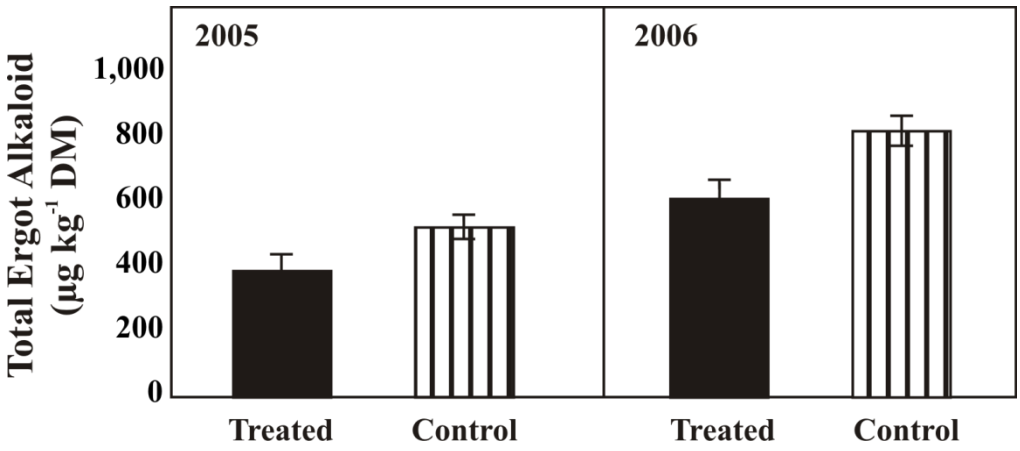

Figure 2.7. Total ergot alkaloid concentration between clethodim-treated and control tall fescue forage in 2005 and 2006 at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Error bars represent 2 SE.

Table 2.1. Average air temperatures and 2 SE for March, April, May and the 30-y average (1977 to 2006) at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist.

Year	Month	Average Air Temperature (°C)		2 SE (°C)	
		High	Low	High	Low
2005	March	13.2	0.1	1.9	1.5
	April	19.8	7.1	1.5	1.9
	May	24.3	10.8	1.5	1.9
2006	March	16.0	2.5	2.5	1.9
	April	23.1	9.3	1.9	1.9
	May	24.9	13.6	1.9	1.9
30-y average	March	13.5	1.0	0.8	0.6
	April	19.0	6.2	0.8	0.6
	May	23.4	11.6	0.6	0.6

Table 2.2. Analysis of variance (ANOVA) results for the clethodim herbicide experiment at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri in 2005 and 2006.

Source	Ergovaline	Total Ergot Alkaloid
	----------(Probability > F)----------	
Year x Treatment	<0.05	<0.05
Year	<0.01	<0.01
Treatment	<0.01	<0.01

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Chapter 3: The Effect of Poultry Litter and Its Chemical Equivalent on Ergovaline and Ergot Alkaloid Concentrations

Ergovaline and ergot alkaloid concentrations in tall fescue forage can be influenced by amount and type of nutrients available to *Neotyphodium* and tall fescue, its host plant. Concentrations may be influenced by type of fertilizer -- poultry litter or chemical fertilizer -- because, as discussed earlier in the literature review, each fertilizer form provides a different combination of plant-available nutrients. Reductions in ergovaline and ergot alkaloid concentration through fertilizer form or fertilizer amount may provide farmers a readily available solution for limiting fescue toxicosis symptoms.

Objective

The objectives of this study were 1) to determine if poultry litter and its chemical fertilizer equivalent impacted ergovaline and ergot alkaloid concentration in tall fescue and 2) if poultry litter and its chemical fertilizer equivalent affected these alkaloids differently.

Methods and Procedures

Experimental Site

The study was conducted 2003 through 2006 on an established, *N. coenophialum*-infected tall fescue pasture (67% endophyte infection) at the Southwest Missouri Research and Education Center (SWC) near of Mt. Vernon, Missouri (37º 04’ N; 93º 53’ W; elevation 351 m). The study was conducted in cooperation with and concurrently on the same plot with McClain (2007) and D. Blevins’ laboratory. The study was initiated in

the autumn of 2003 on a Creldon silt loam, (fine, mixed, active, mesic Oxyaquic Fragiudalf), with a 26% aluminum (Al) saturation. The site represents typical soil conditions for tall fescue pastures in southern Missouri (Hughes, 1982). The study site was low in plant available P (19.8 kg Bray I), and had a pH of 5.3. Spring monthly precipitation is presented in Fig. 3.1 and annual precipitation with the 30-y average is presented in Fig. 3.2. Spring monthly high and low air temperature is presented in Fig. 3.3.

Fertilizer Treatment

During the third week of August 2003, forage was removed from the pasture and 3-m x 7.6-m plots with 1.5-m alleys were established. The field design was a randomized, complete block design with six replicates. Each of the 42 plots received poultry litter, chemical fertilizer (Table 3.1) or no treatment, which served as control plots. Poultry litter was collected from a nearby facility and temporarily stored at the SWC. Poultry litter samples for nutrient analysis were taken immediately prior to application. Poultry litter samples were analyzed by the University of Missouri Soil Testing Laboratory to determine the amount of NPK in the litter and establish rates of chemical fertilizer. Fertilizer treatments were applied in the third week of August 2003. Poultry rates were 0, 2.24, 4.48, and 8.96 Mg ha^{-1}; chemical fertilizer rates, expressed as 1x, 2x, and 4x coincided with the NPK concentrations determined in the poultry litter (Table 3.1). The sources of inorganic nutrients for fertilizer treatments were ammonium nitrate (34-0-0), triple superphosphate (0-46-0) and potassium sulfate (0-0-42). The rates of application of fertilizer were high, and therefore the chemical fertilizer 2x and 4x treatments were split

into 2 and 3 application dates, respectively. All fertilizer treatments were applied in the first year; there were no fertilizer applications in subsequent years (McClain, 2007).

Sample Collection and Laboratory Analysis

Tall fescue forage, 100 g wet weight, was hand harvested by clipping at a 5-cm stubble height. Samples were collected 24 June in 2004, 8 June in 2005 and 7 June in 2006, before seed shatter. Samples were immediately stored at -5°C. After each harvest aboveground biomass was clipped to a height of 10 cm and removed. Frozen samples were freeze-dried, ground with a mill through a 2-mm screen and then ground through 1-mm screen of a cyclone-type mill. Instrumentation included a Pacific Scientific 5000 scanning monochromator (NIRSytems, Silver Spring, MD) with commercial software developed by Infrasoft International (Port Matilda, PA). The reference method for ergovaline analysis was the procedures reported by Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993). Ten g of representative forage samples were analyzed for ergovaline. Spectra for the poultry litter/chemical equivalent study added to a larger dataset including spectra for concurrent studies of clethodim effectiveness, a comparison between calcitic and dolomitic limestone application rates, and phosphorus fertilizer. The calibration and validation statistics for ergovaline included the mean and standard error of calibration 373 ± 53 µg ergovaline kg^{-1} DM; the standard error of cross-validation was 78 µg ergovaline kg^{-1} DM. The squared correlation coefficient for calibration was 0.94, and 1 minus the variance ratio was 0.85.

Two g of representative forage samples were analyzed for total ergot alkaloid concentration with enzyme-linked immunosorbent assay (ELISA) following the

procedures of Hill and Agee (1994) using commercial ELISA test kit (Catalog no. ENDO899-96p; Agrinostics Ltd., Watkinsville, GA). The mean and standard error of calibration were 362 ± 56 µg total ergot alkaloid kg^{-1} DM; the standard error of cross-validation was 99 µg total ergot alkaloid kg^{-1} DM. The squared correlation coefficient for calibration was 0.96, and 1 minus the variance ratio was 0.89.

Statistics Analysis

Ergovaline and total ergot alkaloid concentrations were analyzed as a randomized, complete block with 7 treatments and 6 blocks. A split-plot arrangement of the randomized complete block was utilized where years and blocks were main plots, fertility treatments were sub-plots. Main effects and all interactions were tested. Years and interactions with years were considered as random effects and fertilizer treatment was considered a fixed effect. Results were analyzed with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Preplanned comparisons were used to compare ergovaline concentrations of the control or untreated samples against poultry litter, its chemical equivalent and the combination of the two types of fertilizer used.

Results and Discussion

Ergovaline Concentration

Ergovaline mean forage concentrations ranged from 273 to 652 µg ergovaline kg^{-1} DM. These ergovaline concentrations are typical of fertilized, reproductivly mature, highly endophyte-infected tall fescue pastures (Rottinghaus et al., 1991). Ergovaline concentration in control plots increased each year, from 296 µg ergovaline kg^{-1} DM in

2004 to 431 µg ergovaline kg^{-1} DM in 2005, and to 547 µg ergovaline kg^{-1} DM in 2006. There was a year x fertilizer treatment interaction for ergovaline concentration (Fig. 3.4, Table 3.3).

Depending on the growing conditions, nutrients in manure can be carried over from one year to the next (Blonski et al., 2004, Vandepopuliere et al., 1975), thereby creating a nutrient effect in both the year of application and the following year.

In this study, nutrients affected ergovaline in the year of application only; there was no carryover effect to subsequent years. The reason for the lack of an effect in 2005 and 2006 could have been the excellent growing conditions in the 2004, the year of nutrient application (Fig. 3.2 and 3.3). Rainfall in March, April and May of 2004 was greater than the 30-y averages for those months. As this rainfall created deal weather conditions for forage growth, tall fescue roots would have taken up high concentrations of nutrients (Patton et al., 2007).

In addition to nutrient uptake, nutrients could have been lost by surface runoff or leaching through the soil. This is highly likely for a nutrient such as N, which is water soluble. After the year of application, which was also a year of high precipitation, little N would have been available for plant use in the following years (Blonski et al., 2004, Williams et al., 2001, Fairey and Lefkovitch, 1998).

In 2004, chemical-treated tall fescue forage contained more ergovaline than litter-treated tall fescue ($p<0.004$) (Table 3.4, Fig. 3.5). Chemical-treated forage produced an average of 495 µg ergovaline kg^{-1} DM, and litter-treated tall fescue produced an average of 369 µg ergovaline kg^{-1} DM. The difference was 124 µg ergovaline kg^{-1} DM, which

was a 25% difference. Chemical-treated tall fescue plants would have had immediate access to inorganic fertilizer, whereas, the access by litter-treated tall fescue plants to nutrients, N for example, would depend on the conversion rate of organic N to inorganic N (Sistani et al., 2008) as discussed in Chapter 1. Immediate access to inorganic N would not limit chemical-treated tall fescue forage production like that potentially seen with the use of organic-N-rich poultry litter.

This difference of 124 µg ergovaline kg^{-1} DM between chemical treated and litter treated forage was not only statistically significant, but also biologically important, as reviewed in Chapter 1. Ergovaline concentrations as low as 200 µg ergovaline kg^{-1} DM in Missouri (Cornell et al., 1990) or 475 µg ergovaline kg^{-1} DM in Oregon (Aldrich-Markham et al., 2007) have been reported as thresholds for the emergence of toxicosis behavior symptoms in cattle. Ergovaline concentrations consumed at levels as low as 50 µg ergovaline kg^{-1} DM can instigate physical signs of fescue toxicosis, such as elevated body temperature, at an ambient temperature of 32.2°C (Cornell et al., 1990).

High rates of fertilizer, which included 4.48 Mg ha^{-1} and 8.96 Mg ha^{-1} litter and chemical equivalents treatments, caused greater ergovaline concentrations than the low rates fertilizer, which included control samples and the 2.24 Mg ha^{-1} litter and its chemical equivalent treatment ($p<0.002$) (Table 3.4, Fig. 3.6). High rates caused a mean 468 µg ergovaline kg^{-1} DM; low rates caused a mean 337 µg ergovaline kg^{-1} DM. These results was expected. Increasing rates of N fertilization were reported with increasing ergovaline concentration (Rottinghaus et al., 1991).

Forage of all fertilizer treatments in 2004 contained a greater ergovaline concentration than the untreated tall fescue forage (p<0.016) (Table 3.4, Fig. 3.7). The fertilized forage produced an average 432 µg ergovaline kg^{-1} DM; the untreated, control forage contained 296 µg ergovaline kg^{-1} DM. As discussed above and in Chapter 1, higher ergovaline concentrations would be expected with increasing rates of N and P fertilizer.

Ergot Alkaloid Concentration

Unlike ergovaline concentration, there was no year x treatment interaction for total ergot alkaloid concentration (p>0.05). There were no significant main effects for year or fertilizer treatment (Table 3.3). Mean forage total ergot alkaloid concentrations among 2004, 2005 and 2006 ranged from 548 to 788 µg total ergot alkaloid kg^{-1} DM.

Conclusion

Nutrient management affected ergovaline concentrations in tall fescue. In this study, the effect occurred in the first year only, the year of application. It did not carry over to the next two years. This can be explained by the possibility that nutrients were consumed by the plant, lost to the environment, or both. Such an explanation is supported by the high rate of precipitation in the first year.

High fertilizer rate forage, regardless of nutrient form applied, produced 130 µg ergovaline kg^{-1} DM more than low application rate forage which included the controls. Also, chemical-fertilizer treated forage produced 124 µg ergovaline kg^{-1} DM more than litter-treated forage. These findings fit well with reports that high rates of chemical N fertilizers are likely to increase incidents of fescue toxicosis.

Figures and Tables

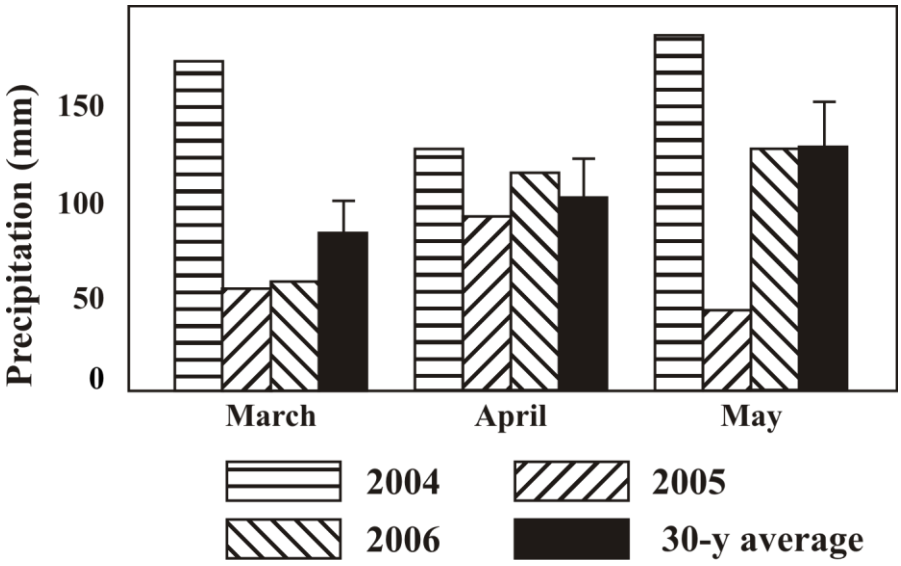

Figure 3.1. Total monthly precipitation for March, April, May and the monthly 30-y average (1977 to 2006) for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Error bars represent 2 SE for the 30-y average.

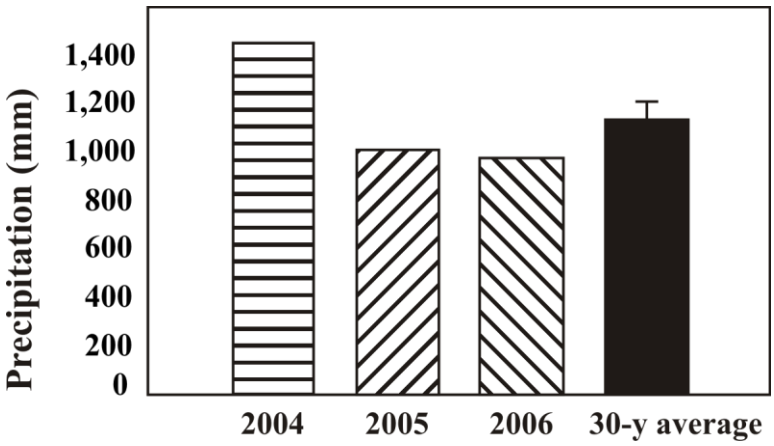

Figure 3.2. Annual precipitation for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Error bar represents 2 SE of the 30-y average.

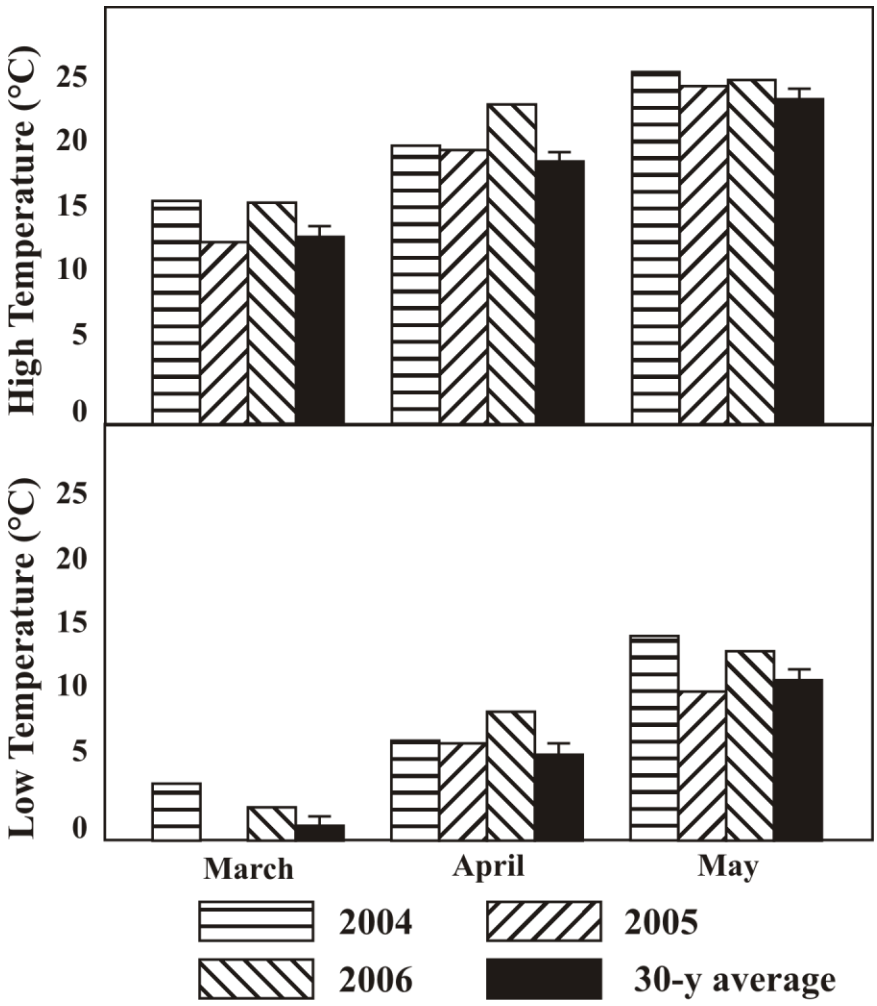

Figure 3.3. Average air temperatures for March, April and May 2004 through 2006, and the monthly 30-y average for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. March, 2005 average low was 0.1 °C. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist. Error bar represents 2 SE of the 30-y average.

Table 3.1. Average air temperature and 2 SE for Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Data provided by Dr. P. Guinan, University of Missouri State Extension Climatologist.

Year	Month	Average Air Temperature (°C)		2 SE (°C)	
		High	Low	High	Low
2004	March	16.1	4.2	1.5	1.9
	April	20.0	7.3	1.7	2.3
	May	25.4	14.6	1.9	1.9
2005	March	13.2	0.1	1.9	1.5
	April	19.8	7.1	1.5	1.9
	May	24.3	10.8	1.5	1.9
2006	March	16.0	2.5	2.5	1.9
	April	23.1	9.3	1.9	1.9
	May	24.9	13.6	1.9	1.9
30-y average	March	13.5	1.0	0.8	0.6
	April	19.0	6.2	0.8	0.6
	May	23.4	11.6	0.6	0.6

Table 3.2. Poultry litter and chemical fertilizer equivalent of poultry litter applied to "Kentucky-31" tall fescue. Listed are rates applied and total nutrient quantities of each treatment.

Nutrient	**Poultry Litter (Mg ha^{-1})**			**Chemical Equivalent**		
	2.24	4.48	8.96	1X	2X	4X
N (kg ha^{-1})	87	175	350	87	175	350
P (kg ha^{-1})	46	92	184	46	92	184
K (kg ha^{-1})	75	154	307	75	154	307

Table 3.3. Analysis of variance (ANOVA) results for the poultry litter and its chemical equivalent experiment conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri.The experiment was initiated in 2003 and harvested in 2004 through 2006.

Source		**Ergovaline**	**Total Ergot Alkaloid**
		----------(Probability > F)----------	
Year x Treatment		<0.05	>0.05
Year	**Treatment**		
2004	Fertilizer	<0.01	0.19
2005	Fertilizer	0.21	0.26
2006	Fertilizer	0.28	0.49

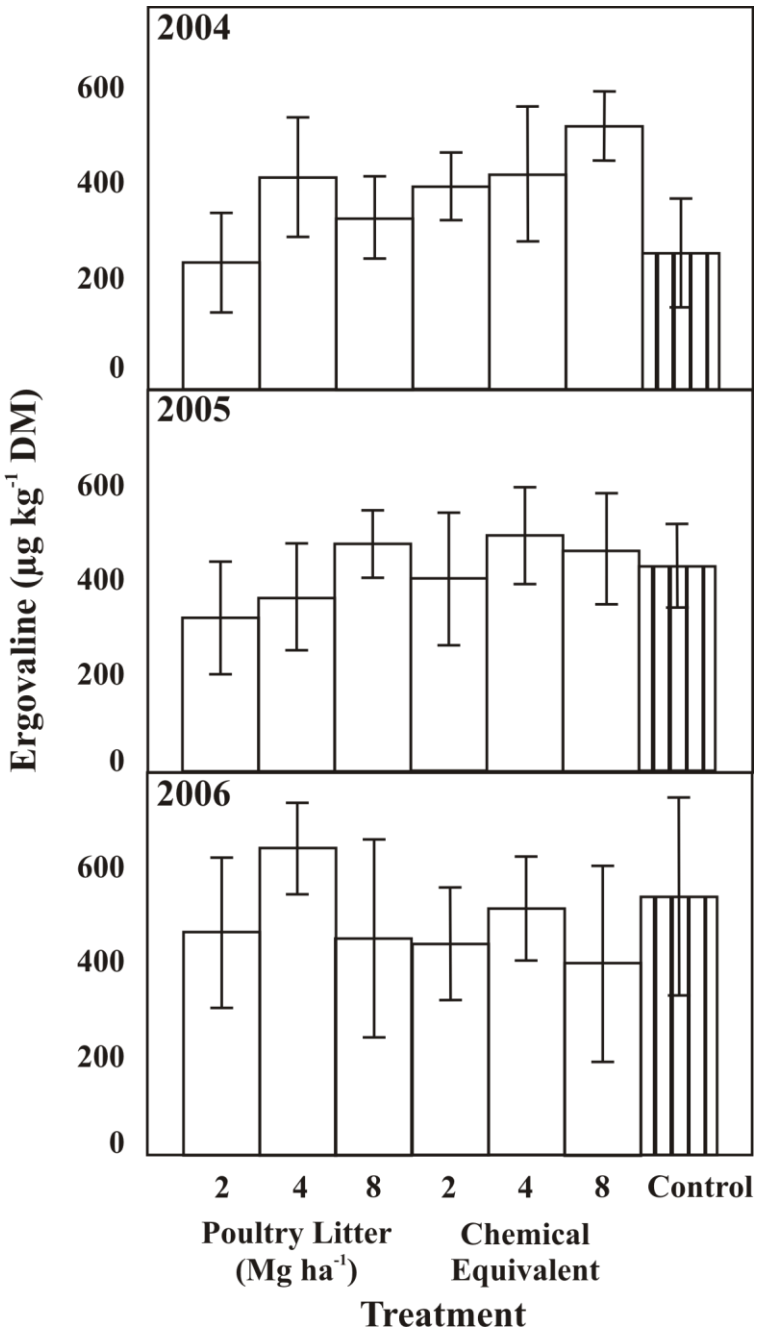

Figure 3.4. Ergovaline concentration among three rates of poultry litter treated, the chemical NPK equivalent treated and the control in 'Kentucky-31' tall fescue forage at the Southwest Missouri Research and Education Center, Mt. Vernon, Missouri in 2004, 2005 and 2006. Error bars represent 2 SE.

Table 3.4. Comparison of ergovaline and total ergot alkaloid concentrations in poultry litter and chemical NPK equivalent treatments applied to 'Kentucky-31' tall fescue forage at the Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Fertilizer was applied in 2003 and forage was harvested 2004 through 2006.

Fertilizer Type	**Ergovaline**			**Total Ergot Alkaloid**		
	2004	2005	2006	2004	2005	2006
Poultry Litter vs. Chemical Equivalent	0.004	NS	NS	NS	NS	NS
Control vs. All Others	0.016	NS	NS	NS	NS	NS
Low Fertilizer (control, 2.24 Mg ha^{-1}) vs. High Fertilizer (>2.24 Mg ha^{-1})	0.002	NS	NS	NS	NS	NS
2.24 Mg ha^{-1} Poultry Litter vs. Control	NS	NS	NS	NS	NS	NS
4.48 Mg ha^{-1} Poultry Litter vs. Control	0.023	NS	NS	NS	NS	NS
8.96 Mg ha^{-1} Poultry Litter vs. Control	NS	NS	NS	NS	NS	NS

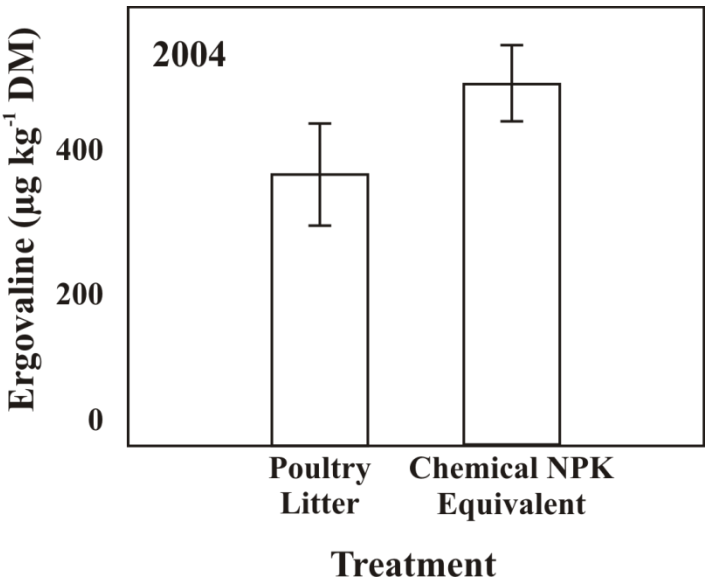

Figure 3.5. Ergovaline concentration in tall fescue forage in 2004 treated with poultry litter and chemical NPK equivalent. Plots were treated in 2003 at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri in 2004. Error bars represent 2 SE.

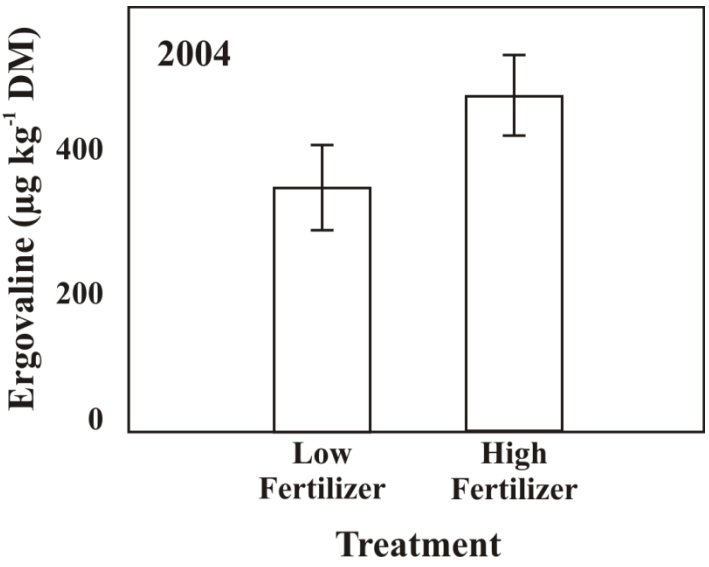

Figure 3.6. Ergovaline concentration in tall fescue in 2004 treated with low fertilizer treated and high fertilizer. Plots were treated in 2003 at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Error bars represent 2 SE.

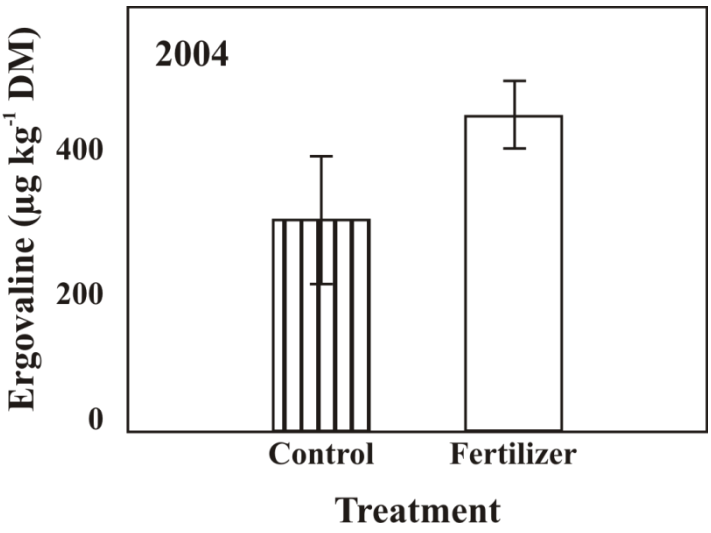

Figure 3.7. Ergovaline concentration in 'Kentucky-31' tall fescue forage in 2004 in the non-treated control and fertilizer-treated forage. Plots were treated in 2003 at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Error bars represent 2 SE.

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Chapter 4: The Effect of Phosphorus on Ergovaline and Ergot Alkaloid Concentrations

Phosphoruse fertilization rate can influence ergovaline and ergot alkaloid concentrations. As discussed earier in the literature review, the effect of P fertilizer on ergovaline and ergot alkaloid is contradictory in the literature and therefore requires more investigation. Specifically, the effect on P on alkaloid concentrations under field conditions is not known.

Objective

The objective of this study is to determine the influence of phosphorus fertilizer application on ergovaline and ergot alkaloid concentration in a tall fescue field.

Methods and Procedures

Experimental Site

The experiment was conducted 2003 through 2006. It utilized an established *N. coenophilum*-infected "Kentucky 31" tall fescue pasture (67% endophyte infection) located at the University of Missouri Southwest Missouri Research and Education Center, outside of Mt. Vernon, Missouri (37º 04' N; 93º 53' W; elevation 351 m). It was conducted in conjunction and concurrently with experiments by McCain (2007) and D. Blevins. The soil was a Creldon silt loam (fine, mixed, active, mesic Oxyaquic Fragiudalf). Bray I P test for soil P was 7.8 kg P ha^{-1} prior to fertilization. The site has a fragipan of dense clay at a depth of 45 to 89 cm and when unmanaged forms a perched water table as high as 45 to 91 cm from the surface from December to April. The

percentage of gravel size rock or larger below the fragipan layer can be as high as 75% of soil composition. This soil overlays limestone karst features representative of conditions found on the Springfield Plateau immediately west of the Ozark Mountains (Hughes, 1982). Spring monthly precipitation (Fig. 3.1) and annual precipitation with the 30-y average (Fig. 3.2) indicated 2004 was a wet year and 2005 and 2006 were dry years. Spring high and low air temperatures is presented in Fig. 3.3 and Table 3.1.

Phosphorus Treatment

During the third week of August 2003, forage was removed from the pasture and 3 m x 7.6 m plots with 1.5-m alleys were established. The field design was a randomized, complete block design with six replicates. Each plot received a single application of 56 kg ha^{-1}, 112 kg ha^{-1} or 228 kg ha^{-1} P as triple super phosphate (0-46-0) or no treatment, which served as control plots. In August of 2003, 2004 and 2005, standing foliage was removed to a height of 10 cm and 112 kg N ha^{-1} as ammonium nitrate (34-0-0) was applied.

Sample Collection and Laboratory Analysis

Tall fescue forage, 100 g wet weight, was hand-harvested by clipping at a 5-cm stubble height. Samples were collected on 24 June in 2004, 8 June in 2005 and 7 June in 2006, which was before seed shatter. Samples were immediately stored at -5 °C. Frozen samples were freeze-dried, ground with a mill through a 2-mm screen and then ground through 1-mm screen of a cyclone-type mill. Between 2 and 3 g of each sample was analyzed by near-infrared (NIR) spectroscopy following the procedures of Roberts et al. (1997, 2005). Instrumentation included a Pacific Scientific 5000 scanning

monochromator (NIR Sytems, Silver Spring, MD) with commercial software developed by Infrasoft International (Port Matilda, PA). The reference method for ergovaline analysis using procedures from Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993). Ten g of representative forage samples were analyzed for ergovaline. Spectra for the phosphorus study was added to a larger dataset including spectra for concurrent studies of clethodim effectiveness, a comparison between calcitic and dolomitic limestone application rates and poultry litter comparison with chemical fertilizer. The calibration and validation statistics for ergovaline included the mean and standard error of calibration 373 ± 53 µg ergovaline kg^{-1} DM; the standard error of cross-validation was 78 µg ergovaline kg^{-1} DM. The squared correlation coefficient for calibration were 0.94, and 1 minus the variance ratio was 0.85.

Two grams of representative forage samples were analyzed for total ergot alkaloid concentraiton with enzyme-linked immunosorbent assay (ELISA) with procedures from Hill and Agee (1994) using commercial ELISA test kit (Catalog no. ENDO899-96p; Agroinostics Ltd., Watkinsville, GA). The mean and standard error of calibration were 362 ± 56 µg ergot alkaloid kg^{-1} DM; the standard error of cross-validation was 99 µg ergot alkaloid kg^{-1} DM . The squared correlation coefficient for calibration was 0.96 and 1 minus the variance ratio was 0.89 .

Statistical Analysis

Ergovaline and total ergot alkaloid concentrations were analyzed as a randomized complete block with 4 treatments and 6 replications. A split-plot arrangement of the randomized complete block was utilized where years and blocks were main plots, fertility

treatments were subplots. Main effects and all interactions were tested. Years and interactions with years were considered as random effects and all others as fixed effects. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

Results and Discussion

Ergovaline Concentration

There was a year effect for ergovaline concentration ($p<0.01$) (Table 4.1, Fig. 4.1). Although there was no P effect at $\alpha = 0.05$ (Table 4.1), the P affect approached significance at $\alpha = 0.15$. There was no year x treatment interaction for ergovaline (Table 4.1), so the main effects of year and P were considered separately.

The year effect can be seen as a decrease in ergovaline from 2004 to 2006 (Fig. 4.1). Ergovaline concentration averaged 713 µg ergovaline kg^{-1} DM in 2004, 513 µg ergovaline kg^{-1} DM in 2005 and 350 µg ergovaline kg^{-1} DM in 2006. By the end of the three-year study, ergovaline concentration had decreased by 51% (Table 4.2).

The annual decrease in ergovaline concentrations may be related to the corresponding decrease of P concentration within the tall fescue plant. In these same study plots, an experiment conducted simultaneously reported that the amount of P in the grass blade decreased between 2004 and 2005 (McClain, 2007). All fertilizer treatments Bray 1 soil P measurements decreased between 2004 and 2005. Soil P in the control plots decreased to 1.1 kg ha^{-1} in 2005.

Low soil P can limit P uptake and *in planta* P movement through intracellular spaces; such a limitation would therefore reduce P available to supply the physiological energy requirements of *Neotyphodium*. With limited soil P, orthophosphates cannot be

stored as a potential nutrient source in the vacuoles of the endophyte. With limited P, therefore, secondary metabolites such as ergovaline would not be produced at high levels (Deacon, 2006, Jenning, 1995).

As stated before, the effect of P on ergovaline concentration in tall fescue was significant at $\alpha = 0.15$. Ergovaline concentrations were lowest in plots fertilized with the low rate of P and highest in plots fertilized with the highest rate of P. In plots receiving 56 kg P ha^{-1}, concentration averaged 448 µg ergovaline kg^{-1} DM across all three years; in those receiving 228 kg P ha^{-1}, concentration averaged 563 µg ergovaline kg^{-1} DM across years. However, the non-treated control plots contained 546 µg ergovaline kg^{-1} DM, which is higher than the low P treatment and equal to the middle P treatment.

The above data suggests the study should be repeated. Because the treatment effect approached significance, a repeat study may show that ergovaline concentration is influenced by P applications, assuming such applications increase plant-available soil P. As discussed in the literature review in Chapter 1, plant available soil P can influence on ergovaline concentration in tall fescue (Malinowski et al., 1998, Azevedo, 1993).

Should the study be repeated, consideration must be given to the level of P in the soil after treatments. In this study, soil P levels may have been too low to influence ergovaline production. After treatments were applied in August 2003, the soils were tested in April 2004; those 2004 tests indicated that Bray I soil P had increased to only 18.4, 26.9 and 77.9 kg P ha^{-1} for 56, 112 and 228 kg P ha^{-1} treatments, respectively. For the control and the two lower P fertilization treatments (0, 56, and 112 kg P ha^{-1}), these Bray 1 P levels were well below University of Missouri (MU) Extension forage

production recommendations. The MU recommendations range from 33.6 to 44.8 kg P ha^{-1} for soil levels (McClain, 2007).

Ergot Alkaloid Concentration

There was no year x P treatment interaction for total ergot alkaloid concentration (Table 4.1). Year and P treatment were considered separately.

As with ergovaline, there was a significant year effect ($p<0.03$) on ergot alkaloid concentration (Table 4.1, Fig. 4.2). However, unlike ergovaline concentration, ergot alkaloid concentration did not decrease over time (Table 4.3, Fig. 4.3). Ergot alkaloid concentration was initially high in 2004, lower in 2005, and higher again in 2006. In 2005, concentration was 11% lower than than in 2004 and 2006 (Table 4.3).

In a companion study conducted in these plots, phosphorus and other cations were measured in tall fescue blades, and the differences reported were attributed to variable precipitation among years (Fig. 3.1 and 3.2) (McCain, 2007). This was especially true of the precipitation in months leading up to harvest. In 2005, precipitation in April and May were far below precipitation in those months in 2004 and 2006 (Fig.3.1).

Lower precipitation, which is associated with low ambient humidity, does not provide an environment conducive to seedhead infection by *Claviceps*, the surface pathogen (Deacon, 2006). It is highly likely, therefore, that the dry conditions in 2005 reduced the incidence of *Claviceps* infection and therefore reduced the production of ergot alkaloids later detected by the ELISA kit.

There was no P fertilizer rate effect ($p=0.27$) on total ergot alkaloid concentration (Table 4.1). For some reason, the highest ergot alkaloid concentration, 716 µg total ergot

alkaloid kg^{-1} DM, occurred in plots that received the lowest P fertilizer rate, 56 P kg P ha^{-1}. The lowest total ergot alkaloid concentration mean, 665 µg total ergot alkaloid kg^{-1} DM, was the untreated control (Table 4.3).

Conclusion

Phosphorus fertilization did not affect ergovaline or ergot alkaloid concentration at α=0.05. However, for two reasons, the effect of P on ergovaline may prove significant in a repeated study. First, the effect of P treatment on ergovaline was significant at α=0.15. Second, the P treatments applied in this study did not result in levels of soil P that would be considered non-limiting; soil P remained below the MU recommended levels for all but the plot receiving the highest rate.

Figures and Tables

Table 4.1. Analysis of variance (ANOVA) results of the P experiment conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Three P rates were applied once to 'Kentucky-31' tall fescue in 2003 and forage was harvested 2004 through 2006.

Source	**Ergovaline**	**Total Ergot Alkaloid**
	----------(Probability > F test)----------	
Treatment x Year	0.60	0.69
Year	<0.01	<0.03
Treatment	0.15	0.27

Table 4.2. Ergovaline concentration among P fertilizer treatments and years of the P experiment conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Phosphorus treatments were applied once to 'Kentucky-31' tall fescue in 2003 and forage was harvested 2004 through 2006.

Phosphorus Treatment	**Ergovaline (µg kg^{-1} DM)**			**Treatment Average‡**
	2004	2005	2006	
0 kg P ha^{-1}	672	563	404	546
56 kg P ha^{-1}	588	455	301	448
112 kg P ha^{-1}	770	525	336	544
228 kg P ha^{-1}	822	509	357	563
Annual Average†	713	513	350	

†Year effect, $p<0.01$

‡Treatment effect, $p=0.15$

Table 4.3. Total ergot alkaloid concentrations among P fertilizer treatments and years of the P experiment conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Phosphorus treatments were applied once to 'Kentucky-31' tall fescue in 2003 and forage was harvested 2004 through 2006.

Phosphorus Treatment	**Total Ergot Alkaloid (µg kg^{-1} DM)**			**Treatment Average‡**
	2004	2005	2006	
0 kg P ha^{-1}	684	649	661	665
56 kg P ha^{-1}	728	676	745	716
112 kg P ha^{-1}	715	657	735	702
228 kg P ha^{-1}	731	591	740	687
Annual Average†	715	644	720	

†Year effect, $p<0.03$

‡Treatment effect, $p=0.27$

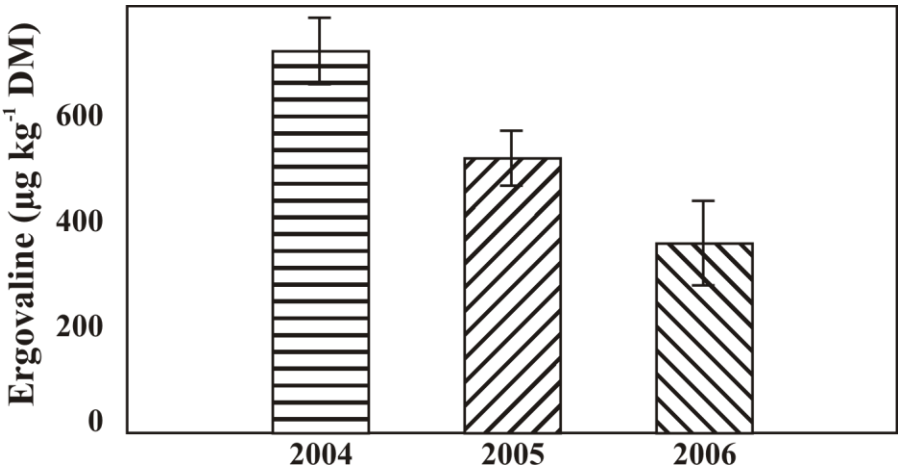

Figure 4.1. Ergovaline concentration among years in 'Kentucky-31' tall fescue forage at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Three P application rates were applied once in 2003 and forage was harvested 2004 through 2006. Error bars represent 2 SE.

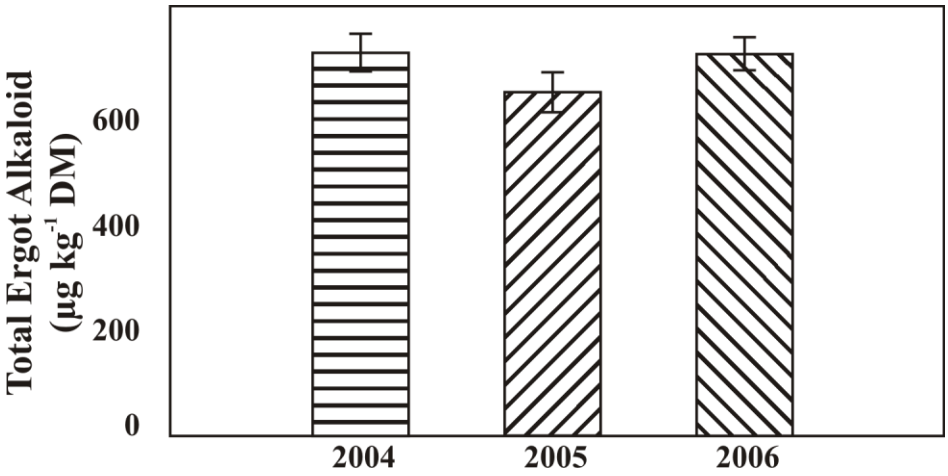

Figure 4.2. Total ergot alkaloid concentrations among years in 'Kentucky-31' tall fescue forage at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Three P application rates were applied once in 2003 and forage was harvested 2004 through 2006. Error bars represent 2 SE.

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Chapter 5: The Effect of Limestone on Ergovaline and Ergot Alkaloid Concentrations

Limestone is a soil conditioner used to increase soil pH and improve forage quality and quantity. Limestone forms include dolomitic limestone [$CaMg(CO_3)_2$] and calcitic limestone ($CaCO_3$). As discussed in Chapter 1, limestone can alter the micro- and macronutrient composition of forage plants, thereby affecting not only the growth but also the composition of tall fescue. Therefore, limestone has the potential to affect ergovaline and ergot alkaloid concentrations.

Objective

The objective of this study was to determine if limestone application would affect total ergot alkaloid concentration and ergovaline concentration of endophyte-infected tall fescue in the immediate years following limestone application.

Methods and Procedures

Experimental Site

The experiment was conducted in 2005 and 2006. It was placed on an established *N. coenophilum*-infected tall fescue "Kentucky 31" tall fescue stand (30% endophyte infection) located on the Southwest Missouri Research and Education Center outside of Mt. Vernon, Missouri (37º 04' N; 93º 53' W; elevation 351 m). This experiment was done in collaboration with E. Hamilton's thesis project (2006) and D. Blevin's laboratory. The soil was a Gerald silt loam (fine, mixed, active, mesic Aeric Fragiaqualf). Initial Bray 1 soil P within the top 15 cm of soil ranged from 32.5 to 46.8 kg P ha^{-1}. Soil

salt pH ranged from 4.9 to 5.3. The site has a fragipan of dense clay at a depth of 51 to 102 cm and, when unmanaged, forms a perched water table above the fragipan from December to April. The percentage of gravel size rock or larger below the fragipan layer can be as high as 75% of soil composition. This soil is formed from a thin mantle of loess or loamy colluvium over cherty limestone residuum (Hughes, 1982). Spring and annual precipitation for this site are presented in Fig. 2.4 and 2.5, respectively.

Limestone Treatment

Calcitic and dolomitic limestone were topdressed on 19 October 2004 at three different rates (Table 5.1) established by the recommended application rate of the Woodward Buffer procedure performed by the Soil and Plant Testing Laboratory, University of Missouri. All plots received 112 kg N ha^{-1}, 73 kg P_2O_5 ha^{-1}, and 258 kg K_2O ha^{-1} in autumn 2004 and in April 2005 as determined from the 14 June 2004 soil test. All plots received 112 kg N ha^{-1} in the autumn of 2005.

Sample Collection and Laboratory Analysis

Tall fescue forage, 100 g wet weight, was hand-harvested by clipping at a 5-cm stubble height. Samples were collected before seed shatter on 8 June 2005 and 7 June 2006. Frozen samples were freeze-dried, ground with a mill through a 2-mm screen and then ground through 1-mm screen of a cyclone-type mill. Instrumentation included a Pacific Scientific 5000 scanning monochromator (NIRSytems, Silver Spring, MD) with commercial software developed by Infrasoft International (Port Matilda, PA). Reference method for ergovaline analysis was by procedures reported by Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993). Ten g of representative forage samples

were analyzed for ergovaline. Spectra for the limestone study added to a larger dataset including spectra for concurrent experiments of clethodim effectiveness, poultry litter and its chemical equivalent fertilizer and a phosphorus fertilizer experiment. The calibration and validation statistics for ergovaline included the mean and standard error of calibration 372 ± 53 µg ergovaline kg^{-1} DM; the standard error of cross-validation was 78 µg ergovaline kg^{-1} DM. The squared correlation coefficient for calibration was 0.94, and 1 minus the variance ratio was 0.85.

Between 2 and 3 g sample were scanned with near-infrared (NIR) spectroscopy following the procedures of Roberts et al. (1997, 2005) for total ergot alkaloid concentration. The reference method for total ergot alkaloid concentration was the enzyme-linked immunosorbent assay (ELISA) following procedures reported by Hill and Agee (1994) using a commercial ELISA test kit (Catalog no. ENDO899-96p; Agrinostics Ltd., Watkinsville, GA). The mean and standard error of calibration were 362 ± 56 µg ergot alkaloid kg^{-1} DM; the standard error of cross-validation was 99 µg ergot alkaloid kg^{-1} DM. The squared correlation coefficient for calibration was 0.96, and 1 minus the variance ratio was 0.89.

Statistical Analysis

Ergovaline and total ergot alkaloid concentrations were analyzed as a randomized, complete block design with 7 treatments and 6 blocks. A split-plot arrangement of the randomized complete block was utilized where years and blocks were main plots, limestone treatments were sub-plots. Main effects and all interactions were tested. Years

and interactions with years were considered as random effects and all others fixed effects. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

Results and Discussion

Ergovaline Concentration

There was no year x limestone treatment interaction for ergovaline concentration (Table 5.2). Data were pooled as year and limestone treatments.

There was no year effect on ergovaline concentration (Table 5.2). In 2005, mean ergovaline concentration was 125 µg ergovaline kg^{-1}DM. In 2006, mean ergovaline concentration was 145 µg ergovaline kg^{-1} DM (Table 5.3). These annual ergovaline concentration means are below the behavior change threshold suggested to be 390 µg ergovaline kg^{-1}DM in Kentucky by Aiken et al. (2009), 475 µg ergovaline kg^{-1} DM in Oregon (Aldrich-Markham et al., 2007) and 200 µg ergovaline kg^{-1} DM in Missouri (Cornell et al., 1990). Physical response in cattle, however, has been measured at concentrations as low as 50 µg ergovaline kg^{-1} DM when cattle were exposed to 32.2 °C (Cornell et al., 1990). Cattle may still physically respond to these low ergovaline concentration when ambient temperatures are high (Cornell et al., 1990) and after prolonged exposure to ergovaline (Klotz et al., 2009).

Ergovaline is primarily associated with *N. coenophailum*-infected tall fescue (Belesky et al., 1988, Porter el al., 1987; Lyons et al., 1986). Endophyte infection level for this stand of tall fescue was 30%. The low infection level may have led to the highly variable ergovaline concentration occurred throughout the site. A more uniform spatial

distribution of endophyte-infected plants found in a higher field infection rate may be required for limestone to influence ergovaline concentrations.

There was no treatment effect on ergovaline concentration (Table 5.2). The untreated control produced the highest mean ergovaline concentration, 175 µg ergovaline kg^{-1}DM. Dolomitic limestone applied at twice the recommended rate contained the smallest mean ergovaline concentration, 90 µg ergovaline kg^{-1}DM (Table 5.3). There was no trend associated with limestone application rate.

Ergot Alkaloid Concentration

There was no year x limestone treatment interaction (Table 5.2). Data were pooled by year and limestone treatment.

There was a significant year effect on total ergot alkaloid concentration (Table 5.2, Fig. 5.1). Total ergot alkaloid concentration was 5% greater in 2005 than in 2006. Tall fescue contained 726 µg total ergot alkaloid kg^{-1}DM in 2005 and 687 µg total ergot alkaloid kg^{-1}DM in 2006 (Table 5.3).

There was no limestone treatment effect on total ergot alkaloid concentration (Table 5.2). The highest mean total ergot alkaloid concentration was the untreated control, 724 µg total ergot alkaloid kg^{-1}DM. The least mean total ergot alkaloid concentration, 697 µg total ergot alkaloid kg^{-1}DM, was from the calcitic limestone applied at 0.5 of the recommended amount (Table 5.3). There was no trend among the limestone treatments.

Conclusions

Neither limestone treatments nor type of limestone affected ergovaline or total ergot alkaloid concentrations in tall fescue forage. Although year did not affect ergovaline concentration, it did affect total ergot alkaloid concentration.

Because this study was conducted on a tall fescue pasture with 30% endophyte infection, it should be repeated on a highly toxic field before using the data to make management recommendations to livestock producers.

Figures and Tables

Table 5.1. Six application rates of limestone material, $CaCO_3$ and $CaMg(CO_3)_2$ that were topdressed onto 'Kentucky-31' tall fescue. The limestone experiment was conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Limestone material was applied in 2004 and harvested in 2005 and 2006.

Limestone Material	**Calcitic Limestone**	**Dolomitic Limestone**
	----------(Mg ha^{-1})----------	
0.5 x Recommended	0.99	1.1
Recommended	1.87	2.2
2.0 x Recommended	3.74	4.4

Table 5.2. Analysis of variance (ANOVA) results for the limestone experiment conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Six limestone application rates were topdressed onto 'Kentucky-31' tall fescue in 2004 and forage was harvested in 2005 and 2006.

Source	Ergovaline	Total Ergot Alkaloid
	----------(Probability > F test)----------	
Treatment x Year	0.26	0.84
Year	0.65	<0.01
Treatment	0.79	0.93

Table 5.3. Ergovaline and total ergot alkaloid concentrations measured in 'Kentucky-31' tall fescue forage among limestone treatments and between years. The limestone experiment was conducted at Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Six limestone application rates were topdressed onto 'Kentucky-31' tall fescue in 2004 and forage was harvested in 2005 and 2006.

Limestone Treatment	**Ergovaline**	**Total Ergot Alkaloid**
---------(Mg ha^{-1})---------	----------(µg kg^{-1} DM)----------	
Dolomitic [$CaMg(CO_3)_2$]		
1.1	128	699
2.2	171	697
4.4	90	718
Calcitic ($CaCO_3$)		
0.99	130	699
1.87	104	723
3.74	157	708
Control	176	724
Year		
2005	125	719
2006	145	676

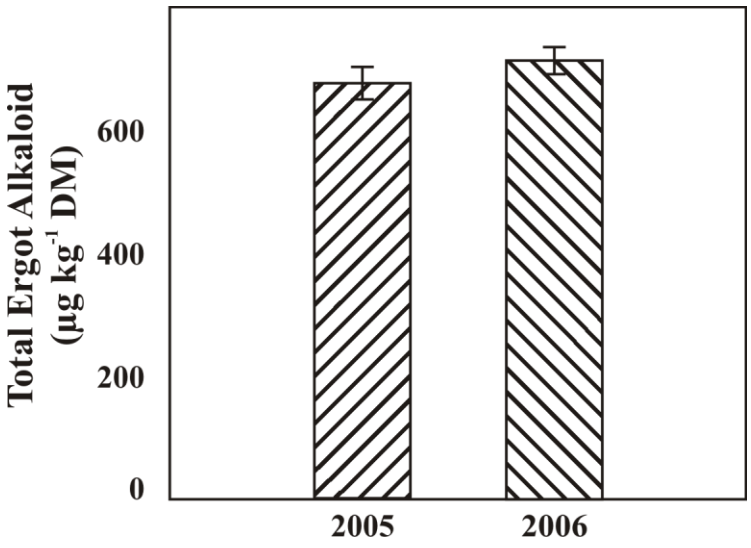

Figure 5.1. Total ergot alkaloid concentration measured in 2005 and 2006 in 'Kentucky-31' tall fescue forage at the Southwest Missouri Research and Education Center, Mt. Vernon, Missouri. Tall fescue was topdressed with dolomitic or calcitic limestone in 2004. Error bars represent 2 SE.

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Chapter 6: Fluctuation of Ergovaline and Ergot Alkaloid Concentrations in Tall Fescue Regrowth

As discussed in the Literature Review of this dissertation, the literature does not contain reports of ergovaline or ergot alkaloid fluctuation in field plants of tall fescue that are regularly clipped. Rather, the literature reports ergovaline or ergot alkaloid concentrations at the beginning, the end, or throughout a grazing experiment. Knowing the fluctuation of ergovaline and ergot alkaloid in clipped fescue throughout the growing season (April to October) could provide insight into the fate of the alkaloids. It would also help producers know how to reduce alkaloid production in a field and therefore offer a solution to improve management of fescue toxicosis.

Objective

The objective of this study was to determine the fluctuations of ergovaline and total ergot alkaloid concentrations in tall fescue regrowth throughout the growing season (April to October) in Georgia, Missouri and South Carolina.

Methods and Procedures

Experimental Sites

This study was conducted in 2006 at three locations: Forage Systems Research Center, Linneus, Missouri (39º 51' N, 93º 8' W, elevation 242 m), University of Georgia Plant Sciences Farm, Watkinsville, Georgia (33º 58'N, 83º 43' W, elevation 293 m) and Clemson University Simpson Research Farm, Pendleton, South Carolina (34 º 3' N, 82 º 42' W, 256 m), hereafter referred to as the Missouri, Georgia and South Carolina site,

respectively. Percentage of forage infected with *Neotyphodium* was at >90% at all three locations. Four 10 x 10 m plots of established fescue were marked in December 2005. Plots were clipped in December 2005 and clippings were discarded. The first harvest was in April 2006, and monthly clippings continued through October.

The Missouri soil was an Armstrong clay loam (Fine, smectitic, mesic Aquertic Hapludalfs). The soil of the site is typical of glacial till developing under tall grass prairie. The soil is now commonly used for cool-season grass hay production and occasional corn and small grain crops. The South Carolina and Georgia site was located on a Cecil clay loam (Fine, kaolinitic, thermic Typic Kanhapludults). This very deep soil is found on ridges and sideslopes of the Piedmont uplands. Cecil series developed from residuum of high-grade metamorphic, weathered felsic and igneous rock. Roughly half of the Cecil series is cultivated with corn, small grains, tobacco and cotton. The other half remains in forest and pasture. Monthly precipitation and average high and low temperature data for all three sites in 2006 are presented in Table 6.1 and 6.2, respectively.

Sample Collection and Laboratory Analysis

Each replicate was hand-clipped at soil surface, and 100-g wet weight subsample was collected. Each site was mowed to a height of 10 cm immediately after harvest. Mowed material was collected and discarded off-site. Samples were immediately placed in storage at -5°C. Frozen samples were freeze-dried, ground with a mill through a 2-mm screen and then through a 1-mm screen of a cyclone-type mill. Ten grams of each sample was supplied for HPLC analysis for ergovaline following the procedures of Rottinghaus

et al. (1991) with modifications reported by Hill et al. (1993). Two grams of each sample was supplied for ergot alkaloid analysis with enzyme-linked immunosorbent assay (ELISA) following the procedures of Hill and Agee (1994).

Statistical Analysis

A mixed model was used to analyze ergovaline and total ergot alkaloid concentrations. Ergovaline and total ergot alkaloid concentrations were analyzed as a randomized complete block with three locations, replications, and 9 harvests. Main effects of month and location and all interactions were tested. Month and interactions with months were considered as fixed effects and location was considered as a random effect. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

Results and Discussion

Ergovaline Concentration

Mean ergovaline concentration among the three locations and months of the growing season (April to October) ranged from 75 to 1,038 µg ergovaline kg^{-1} DM (Table 6.3). There was a significant month x location interaction (Table 6.4), so data were not pooled (Fig. 6.1).

Ergovaline concentration gradually increased from the spring through the summer and peaked in early autumn (Fig. 6.1). Concentrations were nearly identical throughout the spring months in tall fescue from Georgia and South Carolina; they were initially lower in tall fescue from Missouri during this time period. These lower concentrations in Missouri at the onset of spring can be expected, as it simply reflects a lag in the green-up phase due to cooler temperatures in the northern location (Table 6.2).

Ergovaline concentrations in the autumn were highest of all months measured. Autumn concentrations were up to three times those concentrations measured in early spring, depending on location. In Georgia, for example, concentrations for September and October were 1,041 and 916 µg ergovaline kg^{-1} DM, respectively; concentrations in Georgia during April and May were only 265 and 335 µg ergovaline kg^{-1} DM, respectively. Likewise in Missouri, the ergovaline concentration reached their highest levels in autum, 825 µg ergovaline kg^{-1} DM in September and 574 µg ergovaline kg^{-1} DM in October (Table 6.3).

The lowest ergovaline concentrations occurred early in the growing season at all three locations. April in Missouri produced the lowest amount of ergovaline, 75 µg ergovaline kg^{-1} DM. This low concentration in April can be expected, as ergovaline decreases in Missouri tall fescue over the winter months and remains low until well after dormancy is broken (Curtis and Kallenbach, 2007).

The seasonality of the tall fescue growth could be used to explain the rise of ergovaline concentrations. As a cool-season grass, tall fescue can initiate growth at cooler temperatures. Belesky et al. (1988) reported ergovaline concentration increased to antithesis, day 150. Ergopeptine concentration peaked in both the low and high ergovaline producing genotypes in the autumn, after day 300. Two peaks in the growing season of the second year of their study were attributed to environmental or hormonal mediated host-endophyte activity. As stated earlier, Belesky et al. (1988) attributed the summer depression in ergopeptine and ergovaline concentrations as the result of the production of photooxidation metabolites. They suggested oxidation metabolites, in

addition to higher temperatures and high light intensity instigated the breakdown of secondary metabolites such as ergovaline (Belesky et al., 1988; Hellberg, 1957).

Higher temperatures along with lower plant-water status were attributed to the decrease in summer leaf growth in a Missouri field study (Wilhelm and Nelson, 1978). The summer leaf elongation was less, 4.15 mm d^{-1}, when compared to leaf elongation in autumn, 8.54 mm d^{-1}. By comparison, leaf elongation in growth chambers slowed and recovered on a diurnal cycle as the chamber went from light to dark and the air temperature decreased from 20°C to 15°C and returned to light condition and warmed to 20°C. They attributed this twice daily alteration in plants' leaf growth elongation response to its water status (Wilhelm and Nelson, 1978).

Leaf extension carries the growing endophyte away from the apical meristemic region, a nutrient sink. As long as the blade is extending, the endophyte remains within a nutrient sink where nutrients are transported to expanding plants cells and, because of its location, the endophyte. The endophyte applies the nutrients to growth and development and not production of secondary metabolites such as ergovaline. Blades and their endophyte routinely clipped to a height of 10 cm may not have time or the metabolic triggers to store ergovaline in the endophyte vacuoles or lipid droplets (Christensen et al., 2008).

Ergot Alkaloid Concentration

Total ergot alkaloid concentrations among the three locations ranged from 327 to 2,411 µg total ergot alkaloid kg^{-1} DM (Table 6.3). There was a month x location interaction, so pooling across location was not possible (Table 6.4).

There was a bimodal pattern in total ergot alkaloid concentration at all three locations (Fig. 6.2). Concentrations were high in the spring, low in the summer, and high again in the autumn. The biomodal pattern is most clear in the Georgia and South Carolina locations, as these locations revealed a pronounced decrease in the summer months. This decrease is related to the semidormant state of tall fescue, which occurs over a much longer period than occurs in northern Missouri.

Total ergot alkaloid concentration in April was 551 µg kg^{-1} DM at the Missouri location; this was almost a third of concentrations at the Georgia and South Carolina locations for April (Table 6.3). The low concentrations in April at Missouri reflect the low spring low temperatures (Table 6.2). It should be remembered that the Missouri location was near the state border of Iowa, where tall fescue breaks dormancy in March, only one month earlier.

Towards the end of the growing season total ergot alkaloid concentration increased to its highest level (Fig. 6.2, Table 6.3). Total ergot alkaloid concentration in October in Missouri was 2,411 µg total ergot alkaloid kg^{-1} DM, Georgia was 1,972 µg total ergot alkaloid kg^{-1} DM and South Carolina was 2,038 µg total ergot alkaloid kg^{-1} DM.

These concentrations likely reflect alkaloid production from *Neotyphodium.* Without frequent defoliation of the plots, data from the ELISA procedure would reflect alkaloids from the surface fungus, *Claviceps*, which inoculates seedheads. But with defoliation, seedheads are removed. Therefore, without *Claviceps* infection, ergot alkaloid production would likely be from the fungi present in or on the vegetative

material; to date, the literature would indicated the primary fungus would be the tall fescue endophyte, *Neotyphodium*. Ergot alkaloids from the endophyte are found in the leaf blade, sheath and in the culm (Burns et al., 2006, Spiering et al., 2005, Christensen et al., 1997, Keogh et al., 1996, Lyons et al., 1986), not just the seedhead.

This raises the question regarding the seasonal fluctuation of ergot alkaloid production from *Neotyphodium*. Such questions may be answered in part by considering the physiology of tall fescue, how it might affect the physiology of *Neotyphodium*, and how both may affect production of ergot alkaloids. One consideration would be factors that limit grass regrowth, such as dormancy or defoliation; these factors will also limit the extension of the endophyte (Tan et al., 2001, Schmid et al., 2000). It is reasonable to consider that ergot alkaloid production should be limited by mowing, because the energy sinks may be required to support grass regrowth. Also, it may force *Neotyphodium* to prioritize energy into growth instead of secondary metabolism and alkaloid storage that may occur once its host ceases blade extension (Christensen et al., 2002).

Conclusions

Our study reports that regrowth tall fescue, which is achieved by monthly defoliation, contains low to moderate ergovaline concentrations at the beginning of the growing season and through the spring. The highest concentrations occur in the autumn. We attribute the low spring concentrations to removal of seedheads, primarily.
Our study also reports that in tall fescue regrowth total ergot alkaloid concentrations follow a standard bimodal curve for the growing season. The high concentrations occur in the spring and autumn seasons, and the low concentrations occur in the summer. In

northern extremes of the fescue belt, such as Linneus, MO, this summer season is short; therefore, the period of low alkaloid concentrations is short. In the southern extremes, such as Georgia and South Carolina, the opposite is true; the longer summers create long periods in which total alkaloid concentrations are low.

Figures and Tables

Table 6.1. Monthly precipitation in 2006 for Forage Systems Research Center, Linneus, Missouri, Clemson University Simpson Research Farm, Pendleton, South Carolina and University of Georgia Plant Sciences Farm, Watkinsville, Georgia.

Month	**2006 Precipitation (mm)**		
	Georgia	Missouri	South Carolina
January	135	47	112
February	110	1	60
March	146	88	46
April	112	77	58
May	103	67	54
June	102	110	152
July	109	104	41
August	95	135	77
September	95	31	99
October	91	77	104
November	90	51	65
December	97	42	94

Table 6.2. Average high and low air temperature in 2006 for each month at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina.

Month	**Average Air Temperatures (°C)**					
	Georgia		Missouri		South Carolina	
	High	Low	High	Low	High	Low
January	10.9	-0.5	0.9	-0.7	11.3	-0.7
February	11.2	-0.4	7.7	-2.2	14.8	0.9
March	13.8	0.8	5.3	-7.5	12.2	-0.1
April	18.2	4.1	11.5	-0.5	18.2	3.4
May	23.2	8.1	21.4	7.2	25.5	9.9
June	27.0	12.6	22.7	10.9	25.1	10.6
July	30.1	16.8	28.0	16.1	31.1	16.8
August	31.6	19.0	32.2	19.5	32.3	19.8
September	31.0	18.6	29.7	18.9	32.9	20.5
October	27.8	15.7	23.7	9.0	26.8	15.4
November	22.8	9.5	16.6	5.4	22.1	8.2
December	17.8	4.7	12.4	0.7	17.8	2.8

Table 6.3. Ergovaline and total ergot alkaloid concentrations in forage regrowth (April to October) of 'Kentucky-31' tall fescue at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina in 2006.

Month	Georgia		Missouri		South Carolina	
	Ergovaline	Total Ergot Alkaloid	Ergovaline	Total Ergot Alkaloid	Ergovaline	Total Ergot Alkaloid
	----------(µg kg^{-1} DM)----------					
April	283	1,423	75	551	277	1,566
May	335	1,384	322	1,329	396	1,536
June	360	529	324	1,561	348	964
July	448	327	418	1,562	438	1,346
August	555	290	479	857	533	1,475
September	1,038	1,224	825	1,907	795	2,038
October	918	1,972	574	2,411	326	2,346

Table 6.4. Analysis of variance (ANOVA) results for the 'Kentucky-31' forage regrowth during the growing season (April to October) experiment at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina in 2006.

Source	Ergovaline	Total Ergot Alkaloid
	----------(Probability > F test)----------	
Month x Location	<0.001	<0.001
Location	0.0164	0.11
Month	<0.001	<0.001

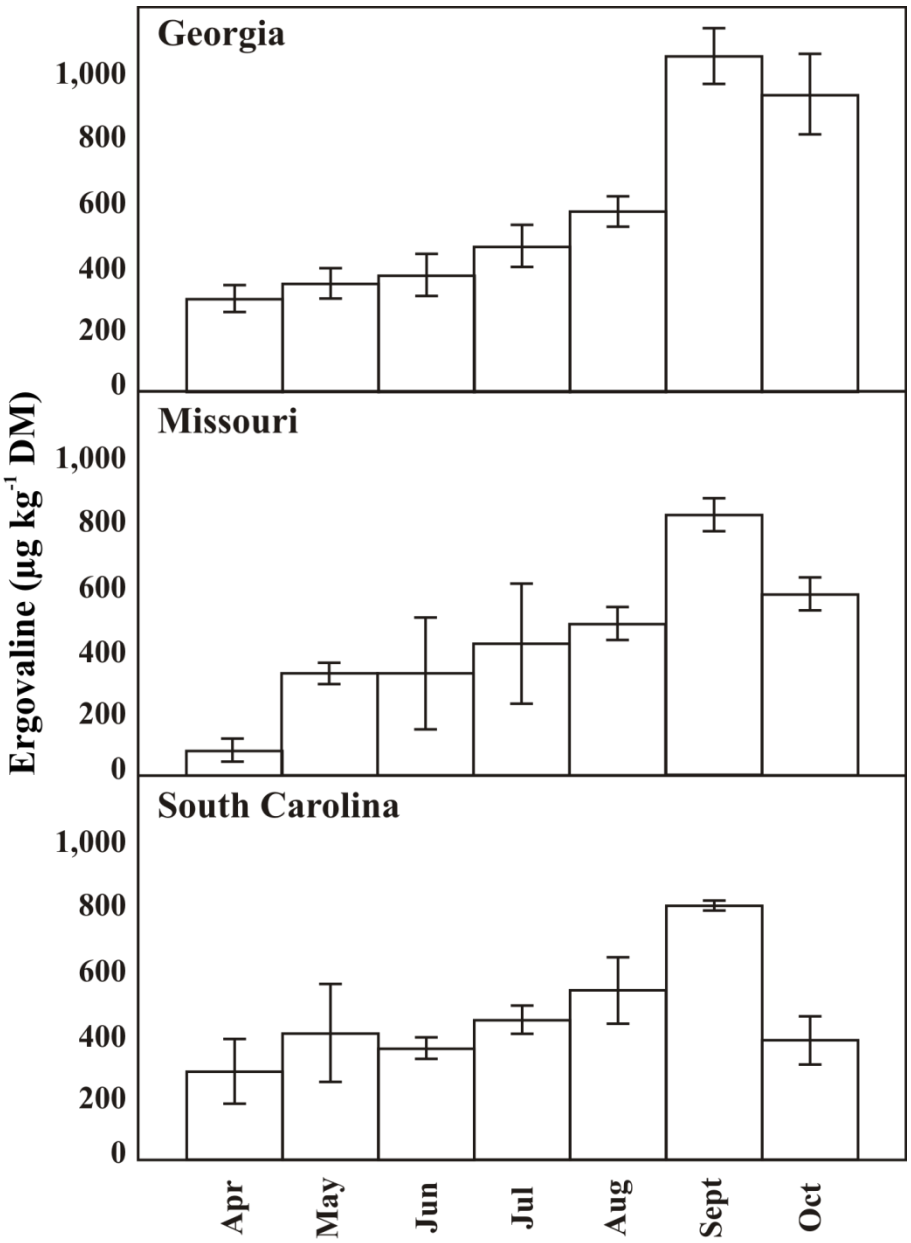

Figure 6.1. Ergovaline concentration in forage regrowth of 'Kentucky-31' tall fescue at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina in 2006. Error bars represent 2 SE.

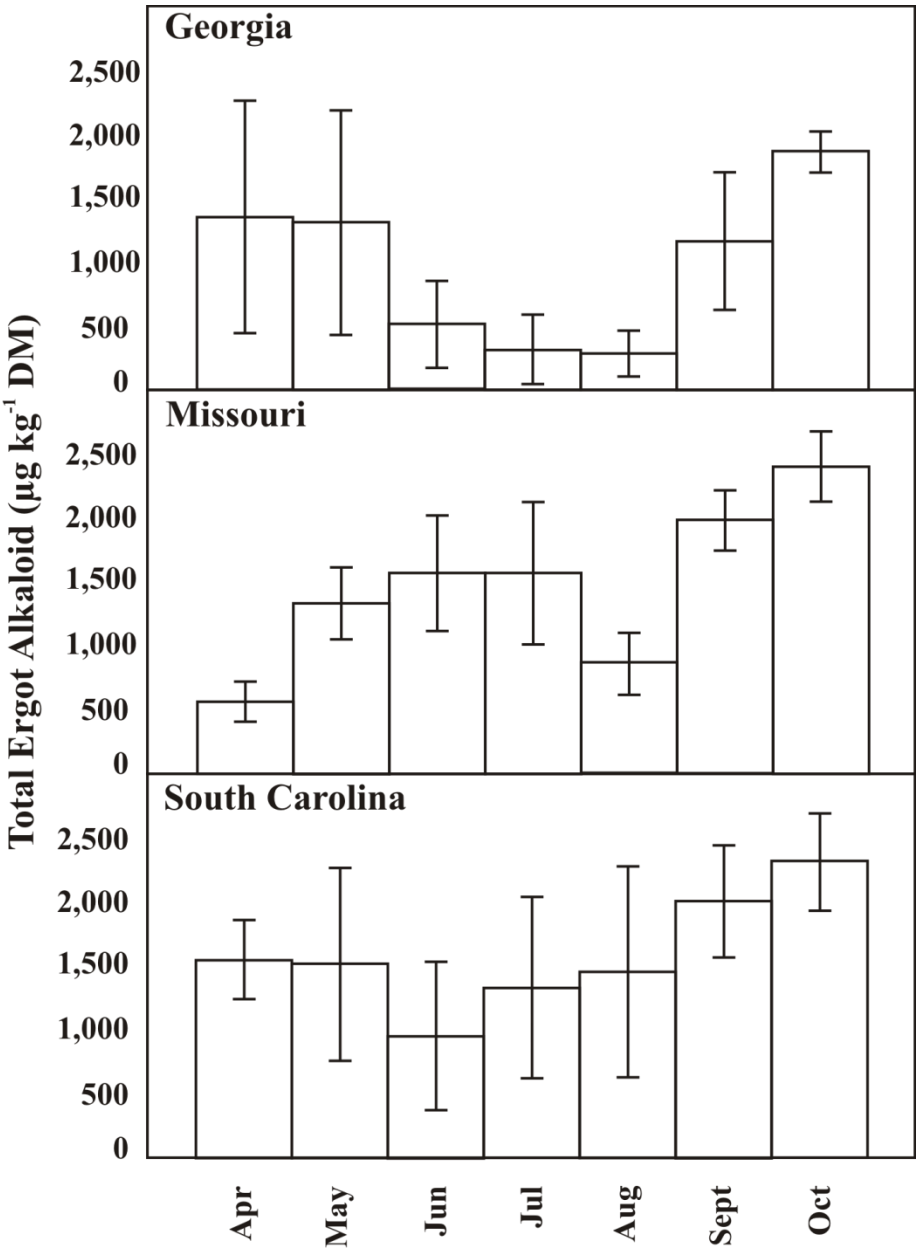

Figure 6.2. Total ergot alkaloid concentrations in forage regrowth in 'Kentucky-31' tall fescue harvested at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina in 2006. Error bars represent 2 SE.

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Chapter 7: Fluctuation of Ergovaline and Ergot Alkaloid Concentrations in Accumulated Growth of Tall Fescue

In the previous chapter, we reported the fluctuation of ergovaline and ergot alkaloid concentration in tall fescue regrowth from Spring through early Autumn, which constitutes the growing season in Missouri for this cool-season grass. That work reported data previously unknown; its importance was the potential effect the data would have on management recommendations for toxic tall fescue pastures.

Also unknown to a lesser extent is the fluctuation of ergovaline and ergot alkaloids in nonharvested toxic tall fescue throughout a calendar year and throughout the fescue belt. Knowledge of this fluctuation in various geographical regions would reveal the potential toxin accumulation in tall fescue forage that is allowed to mature completely before clipping or grazing.

Objective

The objective of this study was to determine the fluctuation of ergovaline and ergot alkaloid concentrations in nonharvested, 'Kentucky 31' tall fescue in nonharvested aboveground tissues over the entire calendar year in Missouri, Georgia, and South Carolina.

Methods and Procedures

Experimental Sites

This study was conducted in 2006. It was established at three locations: Forage Systems Research Center, Linneus, Missouri (39º 51' N, 93º 8' W, elevation 242 m),

University of Georgia Plant Sciences Farm, Watkinsville, Georgia (33º 58'N, 83º 43' W, elevation 293 m) and Clemson University Simpson Research Farm, Pendleton, South Carolina (34 º 3' N, 82 º 42' W, 256 m), hereafter referred to as the Missouri, Georgia and South Carolina site, respectively. Four 10 x 10 m plots of established endophyte-infected tall fescue (>90% endophyte infection) were marked in December 2005. Plots were clipped to a height of 10 cm in December 2005 and clippings were discarded off-site. The first harvest was in January 2006, and monthly clippings continued through December. At the Georgia location, drought conditions limited the herbage available during the months of February, March and June; there was only enough sample for ELISA analysis (see Methods and Procedures below). At the Missouri and South Carolina locations, there were no such limitation in herbage material, and all 12 months underwent complete laboratory analysis.

The Missouri soil was an Armstrong clay loam (Fine, smectitic, mesic Aquertic Hapludalfs). The soil of the site is typical of glacial till developing under tall grass prairie. The soil is commonly now used for cool-season grass hay production and occasional corn and small grain crops. The South Carolina and Georgia site was located on a Cecil clay loam (Fine, kaolinitic, thermic Typic Kanhapludults). This very deep soil is found on ridges and sideslopes of the Piedmont uplands. Cecil series developed from residuum of high-grade metamorphic, weathered felsic and igneous rock. Roughly half of the Cecil series is cultivated with corn, small grains, tobacco and cotton. The other half remains in forest and pasture. Monthly precipitation and average high and low

temperature data for all three sites in 2006 are presented in Table 6.1 and 6.2, respectively.

Sample Collection and Laboratory Analysis

Samples from each replicate were hand-clipped at soil surface, and sample size was 100-g wet weight. Once a month throughout the year, tillers were cut randomly in order to represent the plot which contained both old tall fescue material and, particularly in the fall, new growth. Samples were immediately placed storage at -5°C. Frozen samples were freeze-dried, ground with a mill through a 2-mm screen and then through a 1-mm screen of a cyclone-type mill. Ten grams of each sample was submitted for HPLC analysis for ergovaline following procedures from Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993). Two grams of each sample was supplied for total ergot alkaloid analysis with enzyme-linked immunosorbent assay (ELISA) following the procedures reported by Hill and Agee (1994).

Statistical Analysis

A mixed model was used to analyze ergovaline and total ergot alkaloid concentrations. Ergovaline and total ergot alkaloid concentrations were analyzed as a complete, randomized design with three locations and 4 replicates per 12 harvests. Main effects of month, location and interactions were tested. Month and interactions with months were considered as fixed effects and location was considered as a random effect. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

Results and Discussion

Ergovaline Concentration

A complete picture of ergovaline concentration through a calendar year at all three locations is not possible because drought conditions in Georgia limited forage growth and any subsequent harvest in February, March and June, 2006. Ergovaline concentration measured by the HPLC procedure required more forage material than more than one replicate could provide. This was not a problem at the Missouri or South Carolina locations.

Mean ergovaline concentrations ranged from 75 to 855 µg ergovaline kg^{-1} DM among the three locations over the calendar year (Table 7.1). There was a month x location interaction for ergovaline (Table 7.2).

Ergovaline concentration was low at the beginning of the year in all locations (Fig 7.1). The low concentrations were most apparent in Missouri. This finding agrees with the data reported in the literature. As discussed earlier, ergovaline concentration decreased in a Missouri stockpile study conducted from December through March (Curtis and Kallenbach, 2007), with low concentrations in February and March.

In our study the harvested plant material during these late winter months contained senesced, aboveground material, similar to the stockpile material reported by Curtis and Kallenbach (2007). The plant material was also similar to hay. Research has shown that tissue such tissue contains decreased concentrations of ergovaline. For example, in a Missouri hay study, ergovaline decreased after tall fescue was cut and stored as hay (Roberts et al., 2009). The researchers suggested that the ergovaline

concentration decreased because of the hay curing process prior to baling exposed plant tissue to alkaloid degradation factors such as light (Hellburg, 1957), heat, and oxygen (Fajardo et al., 1995, Garner et al., 1993).

Other points of similarity among the locations was the peak in ergovaline concentration after reproductive maturity, followed by a slight decrease, followed by a second peak of high concentration in ergovaline concentration (Fig. 7.1) in early autumn, and finally a steady decline in concentration through the rest of the calendar year.

A higher concentration in forage in late spring has been reported by Rottinghaus et al. (1991) and Belesky et al. (1988) and was expected. The actively growing tall fescue plant carries the growing endophyte as plant cells extend. The endophyte may be producing ergovaline as it extends with the culm and the developing seedhead (Tan et al., 2001). The growing blade, bladesheath and seedhead act as nutrient sinks, drawing nutrients to developing cells and the endophyte. Seedheads and culms, which are present in late spring, can contain up to 70% of tall fescue's ergovaline concentration (Rottinghaus et al., 1991).

Ergovaline concentrations in the early autumn were high for two reasons—seed that had not shattered and autumn vegetative material. Both of these plant tissues are known to contain high concentrations of ergovaline (Rottinghaus et al., 1991; previous chapter).

The decline in concentrations at the end of the year was expected and typical of that seen in stockpiled tall fescue studies (Curtis and Kallenbach, 2007, Kallenbach et al., 2003). Without further investigation, it is not possible to explain why this decrease

occurs. Possibly ergovaline undergoes addition reactions (Garner et al., 1993) and turnover (Neumann, 1985) in which breakdown products are mobilized during senescence of aboveground material (Mae, 2003). The decrease in ergovaline concentration could be physical, as plant cells freeze then rupture during autumn cold spells, thereby allowing ergovaline to be leaked onto the underlying soil (Kallenbach et al., 2003).

Ergot Alkaloid Concentration

Ergot alkaloid concentration in forage ranged from 275 to 2,502 µg ergot alkaloid kg^{-1} DM (Table 7.1) across months and locations. There was a month x location interaction for ergot alkaloid concentrations (Table 7.2).

Overall, there was little similarity among locations (Fig. 7.2). The following discussion, therefore, will only note these few similarities but will not elaborate further.

In the late summer months, August and September, was the ergot alkaloid concentration pattern similar among locations; concentrations remained stable during these months at all three locations, with South Carolina producing the highest concentrations during the summer.

The greatest ergot alkaloid concentration in forage occurred in the autumn for all locations (Fig. 7.2). Highest concentrations for Georgia were 2,502 µg ergot alkaloid kg^{-1} DM, wich occurred in December; for South Carolina, highest concentrations were also in December at 1,825 µg ergot alkaloid kg^{-1} DM. In Missouri, ergot alkaloid concentration peaked two months earlier at 1,474 µg ergot alkaloid kg^{-1} DM.

In Missouri, total ergot alkaloids followed the same trend as the ergovaline trend (Fig. 7.1 and 7.2). Concentration decreased the first three months of the year, increased in late spring, decreased slightly, then peaked in the autumn. Concentrations declined steadily over the final months of the calendar year.

Conclusions

Although there was a month x location interaction for ergovaline in forage, there were some similarities in the fluctuation of ergovaline concentration over the calendar year among the three locations. Ergovaline concentrations were low in the beginning of the year, increasing through the late spring, decreasing slightly in the summer, peaking in early autumn, and steadily decreasing through the remainder of the year.

Ergot alkaloid concentration in forage, however, contained few similarities among the three locations. Only in Missouri did the forage ergot alkaloid concentration mirror the monthly pattern of ergovaline concentration in forage.

Figures and Tables

Table 7.1. Ergovaline and total ergot alkaloid concentrations in 'Kentucky-31' tall fescue forage harvested at the Forage Systems Research Center, Linneus, Missouri, Clemson University Simpson Research Farm, Pendleton, South Carolina and University of Georgia Plant Sciences Farm, Watkinsville, Georgia for each month of the 2006 calendar year.

Month	**Georgia**		**Missouri**		**South Carolina**	
	Ergovaline†	Total Ergot Alkaloid	Ergovaline	Total Ergot Alkaloid	Ergovaline	Total Ergot Alkaloid
	----------(µg kg^{-1} DM)----------					
January	400	906	255	1,412	431	1,363
February	---	1,229	156	1,299	234	1,224
March	---	917	75	894	564	2,228
April	433	677	120	500	260	1,670
May	426	1,222	334	1,093	372	1,063
June	---	1,718	635	818	555	275
July	618	1,521	549	813	181	575
August	510	1,442	424	839	855	970
September	614	1,398	663	1,068	515	819
October	545	1,714	727	1,474	402	936
November	330	1,245	455	1,241	225	527
December	298	1,825	315	757	160	2,502

† Due to lack of harvested forage, ergovaline concentration for February, March and June will not be reported for Georgia.

Table 7.2. Analysis of variance (ANOVA) results for the forage accumulation experiment conducted at Forage Systems Research Center, Linneus, Missouri, Clemson University Simpson Research Farm, Pendleton, South Carolina and University of Georgia Plant Sciences Farm, Watkinsville, Georgia in 2006.

Source	Ergovaline	Total Ergot Alkaloid
	----------(Probability > F test)----------	
Month x Location	<0.001	<0.001
Location	0.469	0.003
Month	<0.001	<0.001

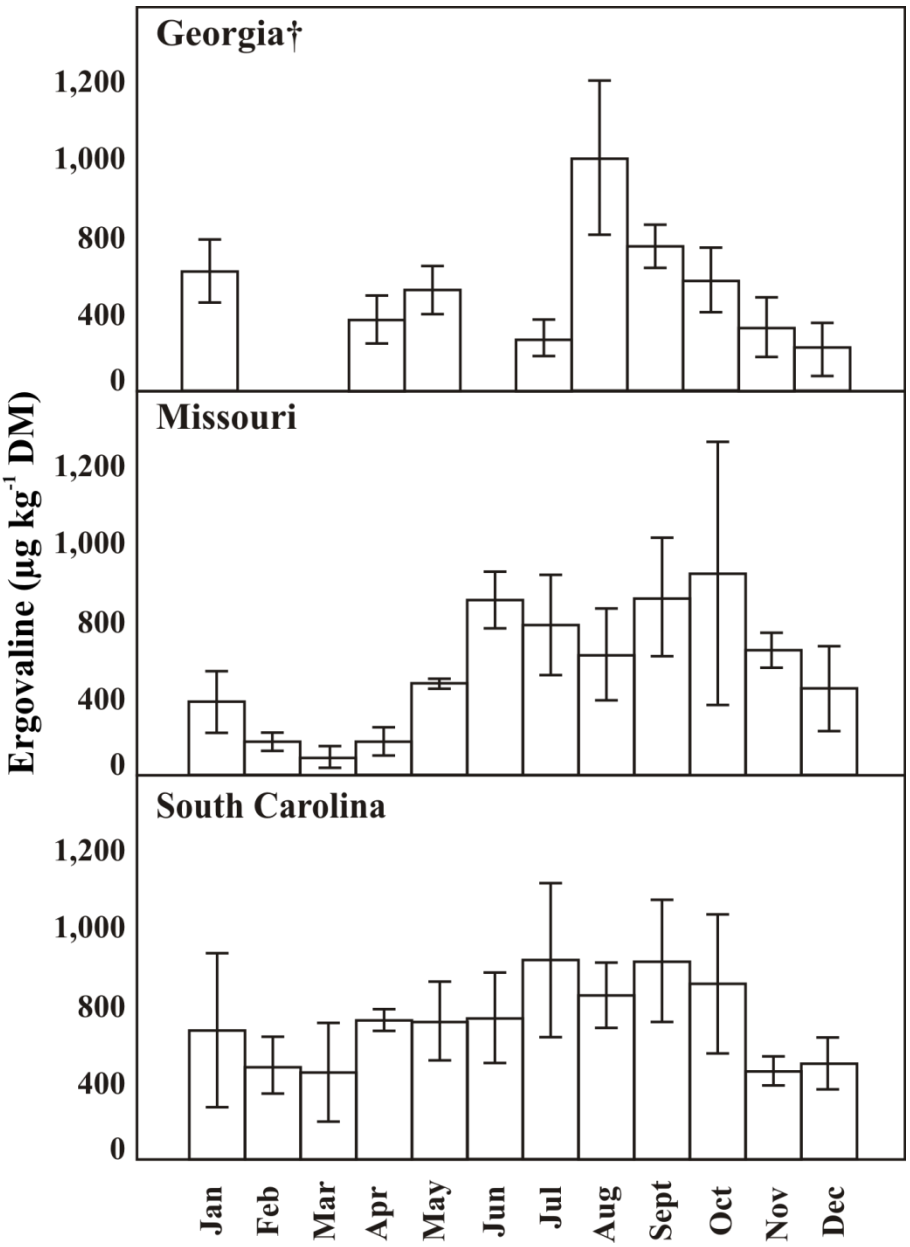

Figure 7.1. Ergovaline concentrations measured in accumulated 'Kentucky-31' tall fescue forage harvested in 2006 at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina. Error bars represent 2 SE.

† In Georgia, ergovaline concentration for February, March and June are not reported due to lack of adequate forage to harvest in 3 of 4 replications.

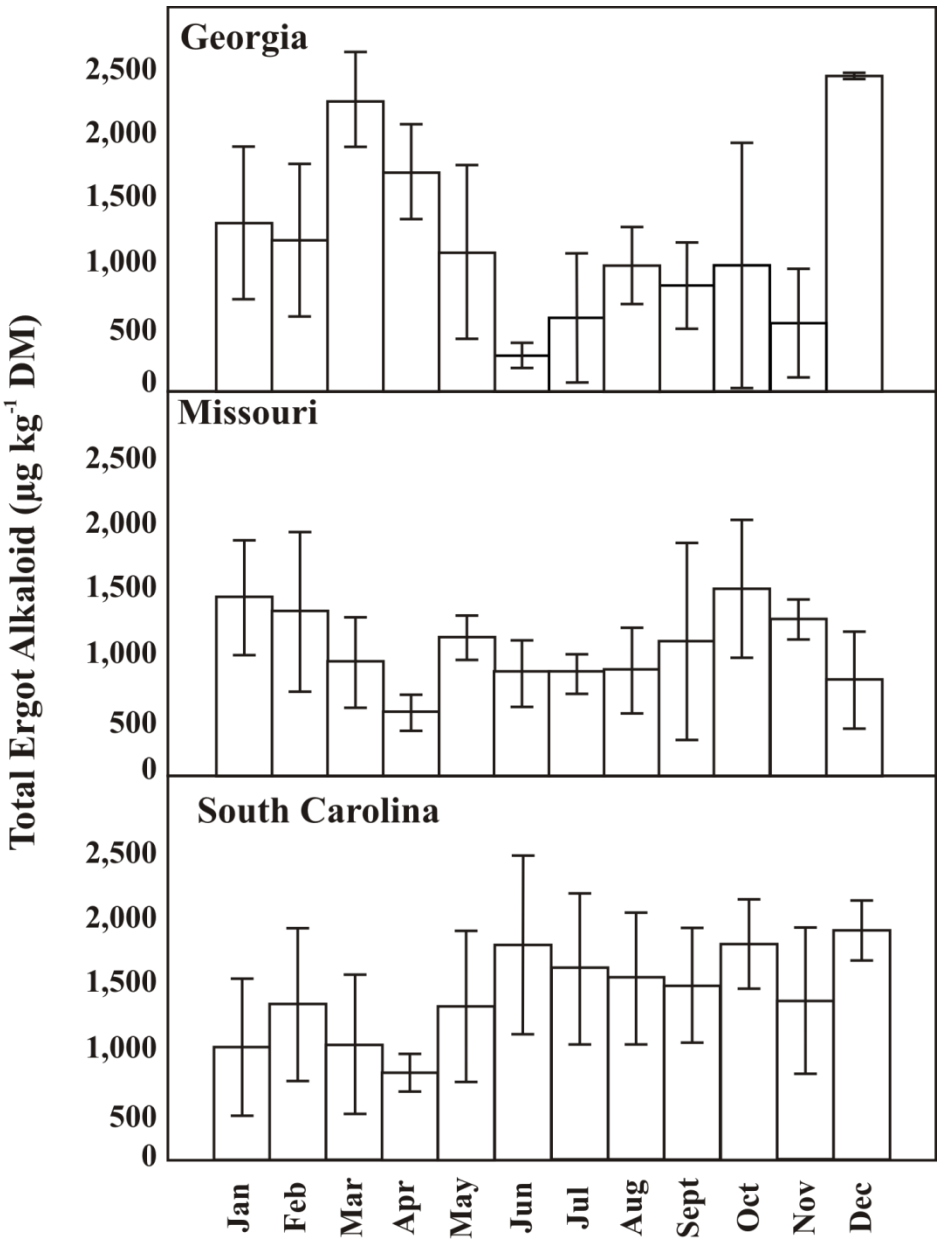

Figure 7.2. Total ergot alkaloid concentration in 'Kentucky-31' tall fescue forage harvested at University of Georgia Plant Sciences Farm, Watkinsville, Georgia, Forage Systems Research Center, Linneus, Missouri, and Clemson University Simpson Research Farm, Pendleton, South Carolina in 2006. Error bars represent 2 SE.

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Chapter 8: Economic Analysis of Reduced Cow Herd Calving Rate and Stocker Gain

As discussed in Chapter 1, the economic impact of fescue toxicosis on livestock enterprises is the result of lower rate of gain and reduced calving rates. For example, beef production stocker animals grazing endophyte-infected tall fescue produce roughly half the average daily gain compared to stockers grazing non-toxic forage. Another example is calving rates of less than 50% for a cow herd grazing toxic tall fescue and receiving no grain supplementation. The economic impact of endophyte-infected tall fescue was estimated to reduce the conception rates of 8.5 million beef cows nationally (Hoveland, 1993). This economic loss, locally and nationally, is associated with exposure to toxic endophyte-infected tall fescue and the compounds ergovaline and other ergot alkaloids.

Also as discussed in Chapter 1 and mentioned above, the reductions in gain and calving rate are associated with ergot alkaloids, particularly ergovaline. The research in chapters 2 through 7 showed that ergovaline concentrations in 'Kentucky-31' tall fescue could be affected by nutrient management and herbicide treatment. What was not shown, however, was the additional cost of each management tool--herbicide, clipping or fertilizer rate--in relation to the additional income from alleviating reduced calving rate and stocker gains due to fescue toxicosis.

As discussed earlier in the results of individual experiments, forage management can reduce ergovaline and ergot alkaloid concentrations. The question now is, do these management schemes pay for themselves, or would accepting lower calving rate or ADG be more profitable? Each forage management practice, whether it is herbicide

application, fertilizer amount, fertilizer form or clipping regime, has a known cost and a predictable return. The forage management practice with the highest net returns per cow or per ac could provide a farmer with the information to choose which practice to use, depending on the farm's circumstances.

Objective

The objective of this chapter is to quantify how the nutrient, herbicide, and clipping practices, particularly as reported in earlier chapters of this dissertation, might impact the economics of a beef cattle enterprise.

Methods and Procedures

A literature review was conducted to identify the extent of the reduction in calving rate and ADG in cattle due to fescue toxicosis (Fig. 8.1). The literature review suggested a linear relationship between ergovaline concentration and endophyte infection level (Drewnodki et al., 2009, Thompson et al., 1993, Washburn and Green, 1991, Gay et al., 1988) (Table 8.1). It reported that a linear relationship appeared to exist between endophyte infection level and spring calving rate. It also reported a linear relationship between endophyte level and stocker ADG (Thompson et al., 1993). The culmination of such reports suggested a linear equation could be constructed to represent reduced calving rate and ADG that was the result of ergovaline concentration.

The equations to be used in this analysis are as follows:

Equation 8.1

f(4.25 µg ergovaline kg^{-1} DM /percent endophyte infection) = -0.375 % in calving rate and

Equation 8.2

$$f(4.25\ \mu g\ \text{ergovaline}\ kg^{-1}\ DM\ /\text{percent endophyte infection}) = -1.3\ g\ \text{average daily gain for summer grazing}$$

The ergovaline concentration in harvested forage was used to predict calving rate and average daily gain (Fig. 8.2). In the analysis of clipping, a range of ergovaline concentration from June forage was used to predict calving rate.

Cow-calf costs for this study were adapted from the Missouri Beef, Cow-Calf Enterprise with Spring Calving budget for 2009. The partial budgets used in this study took into account the estimated relationship between ergovaline concentration and reduction in herd calving rate and ergovaline concentration and stocker ADG.

This study's partial budgets estimated the return per cow, the amount of money required to cover fertilizer treatment, herbicide or clipping cost, and marketing costs (Tables 8.2, 8.3, 8.4 and 8.5). Except for ergovaline concentrations, measurements are in English units, not metric, in order to discuss the practical aspects of the practices.

A partial budget is a tool that quantifies and compares the financial effects of alternative technologies. It allows for the analysis of changes in one management component. All other farm practices remain the same (Table 8.2). A partial budget allows for identification of economic weaknesses of proposed management changes (Almi and Manyong, 2000).

Costs of listed management practices were estimated from fertilizer and herbicide prices quoted from the Missouri Farmer Association (MFA) on 10 November, 2009 and are as follows. Cost of N was $\$0.45\ lb^{-1}$. Cost of P was $\$0.29\ lb^{-1}$. Cost of K was $\$0.45\ lb^{-}$

[1]. Cost of custom driving and spraying of dry fertilizer topdressed on pasture was $4.63 ac^{-1}. The cost of herbicide clethodim 'Select Max' was $150.00 gal^{-1}, applied at a rate of 2.56 oz with 1 qt crop oil ac^{-1}, which translates to a cost of $3.00 ac^{-1}. The cost of custom driving and spraying the herbicide was $5.50 ac^{-1}. Cost of clipping was the listed mid-price in the range of custom rates for Missouri farm services, which was $13.00 ac^{-1} (Plain et al., 2009).

The listed management costs and returns of this study were based on the following estimations. The cost of fertilizer per cow was estimated as the product of cost of lb fertilizer, multiplied by amount applied, multiplied by 200 ac, and then divided by 42 cows (Table 8.4). Marketing cost was estimated by multiplying the return per cow by 2.5% (Table 8.5). Cost per cow was the sum of fertilizer or herbicide cost and marketing cost. Return per cow was gross returns per cow minus the sum of listed cost per cow. Return per ac was the gross returns per cow, multiplied by the number of mother cows, and then divided by the number of ac (Table 8.7).

A herd size of 50 animals on 200 ac was selected as representing a 2007 USDA Ag Census average Missouri beef herd and beef farm. The herd contained 2 bulls, 6 replacement heifers and 42 cows, for a total of 50 animals. A herd's calving rate was estimated from the measured ergovaline concentration of a nutrient or herbicide treatment and applied to the 42 cows.

The stocker herd was 200 animals, 100 steers and 100 heifers, grazing 200 ac. Animals were assumed to be purchased when steers weighed 580 lb and heifers weighed 560 lb. Animals were sold when steers weighed 815 lb and heifers weighed 771 lb, for an

average gain 235 lb steer^{-1} and 211 lb heifer^{-1}, regardless how many days it took to obtain the final weight (Dhuyvetter et al., 2009).

Results and Discussion

Calving Rate

As discussed earlier in Chapter 3, which reported the results of the poultry litter and its chemical NPK equivalent fertilizer experiment, only in year 1 was there an difference in ergovaline concentration between poultry litter treatments and chemical NPK equivalent treatments. Tall fescue fertilized with poultry litter contained less ergovaline, 369 µg ergovaline lb^{-1} DM, than the chemical NPK fertilizer, 495 µg ergovaline kg^{-1} DM (Fig. 3.5). Tall fescue fertilized with poultry litter produced a predicted calving rate of 67%, whereas tall fescue treated with chemical NPK fertilizer had a predicted calving rate of 56% over one year.

The highest predicted calving rate among the fertilizer treatments, 69%, was from forage of the 2 t ac^{-1} poultry litter treatment (Table 8.5). The lowest predicted calving rate, 55%, was from forage of the 8 t ac^{-1} chemical NPK equivalent. Predicted calving rates varied among fertilizer treatments even when the ergovaline concentration was not statistically different. The magnitude of variation in these calving rates would have a significant economic impact on a cow production enterprise.

The predicted gross return per cow for the 2 t ac^{-1} poultry litter treatment, $500.67, was the highest of any poultry litter or chemical NPK equivalent treatment. The gross return minus listed cost per cow of the 2 t ac^{-1} poultry litter, $107.20, and return per ac, $22.51 (Table 8.5), was the highest return per cow or return per ac of the non-treated

control, poultry litter or chemical NPK fertilizer treatments. The non-treated control was predicted to produce a calving rate of 63%, generate the highest gross return minus listed cost per cow, $453.11 and the highest return per ac, $95.15 of the partial budget without considering all costs (Table 8.3, 8.5, and 8.6).

Historically, the listed cost, whether it was poultry litter or its chemical NPK equivalent, would determine if a practice were economically feasible. The cost of a ton of poultry litter delivered and spread used in this analysis was $40. The chemical NPK equivalent cost was a sum total of $81.58 (Table 8.2). Fertilizer cost in this analysis was compared with the predicted calving rate of the forage receiving the fertilizer; now, not only the cost of fertilizer can be taken into consideration but also the potential income gained or lost because of changes in ergovaline concentration and calving rate associated with fertilizer application. The lowest fertilizer cost and highest return per cow for the poultry litter experiment with a predicted 63% calving rate, $453.11, was estimated for the non-treated control. This is simply because there was no fertilizer cost. Nevertheless, fertilizer cost to a farm should not be eliminated. Fertilizer is known to be an important variable for increased forage production and required to meet the forage quality and quantity needed to sustain a cow herd. Forage production was not a factor under consideration in this analysis. Fertilizer effect on calving rate was what was considered; increasing the amount of fertilizer applied, either as poultry litter or chemical NPK equivalent, did not increase calving rate or return per cow.

Phosphorus application, as stated in Chapter 4, did not statistically effect ergovaline concentrations. However, 50 lb P ac^{-1} produced a simulated calving rate 8 to

10% points greater than the other P treatments; this is because its ergovaline concentration was numerically lower, 96 to 115 µg ergovaline kg^{-1} DM, than concentrations from the other P treatments (Table 8.7). Although P treatment did not have a significant effect on plant-fungal toxin production, the calving rates of cows consuming the forage were predicted to reflect the changes in the toxin load. The non-treated control produced the highest estimated return per cow, $388.87, of the P fertilizer experiment. The non-treated control predicted a 52% calving rate because of the 546 µg ergovaline kg^{-1} DM.

Of the three studies analyzed in the economic analysis, the herbicide-treated tall fescue in both 2005 and 2006 produced the highest estimated calving rates, which were 73% in 2005 and 77% in 2006 (Table 8.8.). The no-herbicide control predicted calving rates of 57% in 2005 and 17% in 2006. The 2006, no-herbicide control had the lowest predicted calving rate (17%) among all treatments in the economic analysis, and this low rate reflected the high ergovaline concentration, 942 µg ergovaline kg^{-1} DM. The low calving rate predicted by the no-herbicide control may not reflect reality, because cattle will cease grazing and self-limit toxin consumption when forage is this toxic. Therefore, tall fescue forage containing more than 790 µg ergovaline kg^{-1} DM may not produce a spring calving rate lower than 30%, as reported in experimental conditions (Table 8.1).

The low ergovaline concentrations of the herbicide-treated tall fescue and resulting higher calving rates can be explained by the physiological suppression of seedheads by clethodim. Ergovaline concentrations among the other studies included treatments that did nothing to control seedhead production. Ergovaline is highly

concentrated in seedheads and culms (Rottinghaus et al., 1991). Without seedheads, the ergovaline concentration was possibly decreased by up to 70% and limited to the leaf blades.

As discussed in Chapter 6, clipping once per month maintained a relatively low ergovaline concentration, although it would increase toward the end of the growing season. The cost of clipping, \$13.00 ac^{-1} $trip^{-1}$, was summed over the seven months of this alkaloid fluctuation experiment (Table 8.9.). The lowest ergovaline concentration, 325 µg kg^{-1} DM, predicted a higher calving rate (71%) when compared to the non-clipped forage in the adjacent plots. However, clipping did not produce the highest return per cow, \$66.50, or highest return per ac, \$13.97. Recall, the non-clipped forage accumulation experiment discussed in Chapter 7 occurred alongside the regrowth experiment. These non-clipped plots provided a comparison treatment for contrast against clipped forage in this economic model. Non-clipped forage was estimated to have produced an ergovaline concentration of 550 µg kg^{-1} DM, the lowest calving rate, 51%, had the highest return per cow, \$383.05, and return per ac, \$80.44. The cost of monthly clipping could not pay for the increase in cow calving rate.

Average Daily Gain

Average daily gain predicted for stockers consuming forage from the poultry litter and chemical NPK equivalent experiment was predicted to be 1.6 and 1.7 lbs d^{-1} (Table 8.10). The ADG of 1.7 lb d^{-1} in the 2 t poultry litter ac^{-1} treatment was the highest ADG among all nutrient treatments. The ADG of the remaining two poultry litter treatments and all three chemical NPK equivalent treatments were estimated to be 1.6 lb d^{-1}.

Average daily gain influenced the number of days on grass. The ADG needed to reach the sale weight of 815 lb for steers and 771 lb for heifers was as short as 138 d when steers gained 1.7 lb d^{-1} and heifers gained 1.5 lb d^{-1}. The ADG of the remaining poultry litter and chemical NPK equivalent treatments required an estimated 147 d to reach the desired sales weight, as stockers gained 0.1 lb d^{-1} less each day.

The cost of time required for the animal to obtain the desired sale weight was determined by for ADG in the forms of loan interest and labor (Fig. 8.3). Cost of time as loan interest at 6% on the purchase of the stocker animals was estimated to be \$0.105 $animal^{-1}$ d^{-1}. Cost of labor was predicted to be \$0.0068 $animal^{-1}$ d^{-1}. Should an animal gain the desired 2 lb d^{-1}, over a period of 118 d, that animal would acrue a cost of \$12.39 $animal^{-1}$ and \$2,478 for the 200-animal herd grazing 200 ac. Cost of time required for animals to obtain the sale weight when grazing the fertilizer treatments was greater than the optimal ADG of 2 lb d^{-1}. Cost of animals grazing the control, the no fertilizer application, a predicted \$15.43 $animal^{-1}$, would indicate it would be a preferred management strategy. However, not fertilizing cannot be viewed as a sustainable grazing practice. Cost of time because of less than optimal ADG cannot be paid for by avoiding the cost of fertilizing.

Average daily gain of steers was predicted to be the same for the 0, 50 P lb, 100 P lb, and 200 P lb ac^{-1} treatments, 1.6 lb d^{-1} (Table 8.10). The predicted ADG of the 0 lb P ac^{-1} treatment or non-treated control was the same as all P treatments and cost nothing. The ADG of animals grazing the non-treated control may appear to be more cost-effective because of lack of fertilizer cost; however, not fertilizing cannot be regarded as

a sustainable forage management practice in regions with low soil P. The lack of variation in ADG also means the cost of time was similar among non-treated control and P treatments, \$16.43 animal^{-1}.

Average daily gain between clethodim-treated tall fescue forage was predicted to be the highest of all analyzed treatments, 1.8 lb d^{-1} in the shortest time period, 131 d (Table 8.10). The ADG of the non-treated forage in 2006 was the lowest of all treatments, 1.2 lb d^{-1}, and was predicted to require 195 d of grazing to obtain the sale weight of 815 lb in steers. The ADG of the non-treated forage increased the cost of time for the animal to reach sale weight, \$21.80 animal^{-1}. The lowest ADG had the highest cost of time per animal and herd (Fig. 8.3). The ADG of the clethodim-treated forage was estimated to produce the lowest cost of time over 131 d, \$15.43 animal^{-1}, and the highest return, \$136.21 animal^{-1} and ac^{-1} (Table 8.11). Of all the fertilizer, clipping and clethodim experiments in this analysis, ADG of the clethodim-treated forage showed it to be the best, sustainable technological solution to managing ergovaline concentrations.

Conclusions

Nutrient, herbicide, and clipping management can be used to control the level of ergovaline concentrations. These management practices can have an influence on a herd's calving rate and resulting income (Table 8.5). Of the three nutrient and herbicide practices analyzed, the herbicide treatment was predicted to produce the highest calving rate and highest return per cow and per acre (Table 8.5). Poultry litter produced the higher return compared to chemical equivalent NPK fertilizer. Chemical fertilizer management could potentially provide more income per cow depending on the cost of

fertilizer. The low P fertilizer rate, 50 P ac^{-1} was predicted to provide the highest calving rate among the P treatments. Although the management decision to not fertilize as replicated by the nutrient studies non-treated controls may appear economically desirable, such a practice would not promote sustainable forage production.

Nutrient, herbicide, and clipping management of ergovaline concentrations can also influence stocker ADG. Management of ergovaline concentrations with clethodim produced the highest steer ADG, 1.8 lb d^{-1}, with the lowest number of days on grass, 131 d. Management also influenced the cost of time in the forms of loan interest and labor. Management of ergovaline by clethodim was predicted to produce the lowest cost of time per animal and per ac. Average daily gain was not as sensitive to ergovaline concentration in tall fescue forage produced by nutrient and herbicide treatments as herd calving rate.

Figures and Tables

Figure 8.1. Steps involved in estimating calving rate and predicting the economic outcome based on ergovaline concentrations.

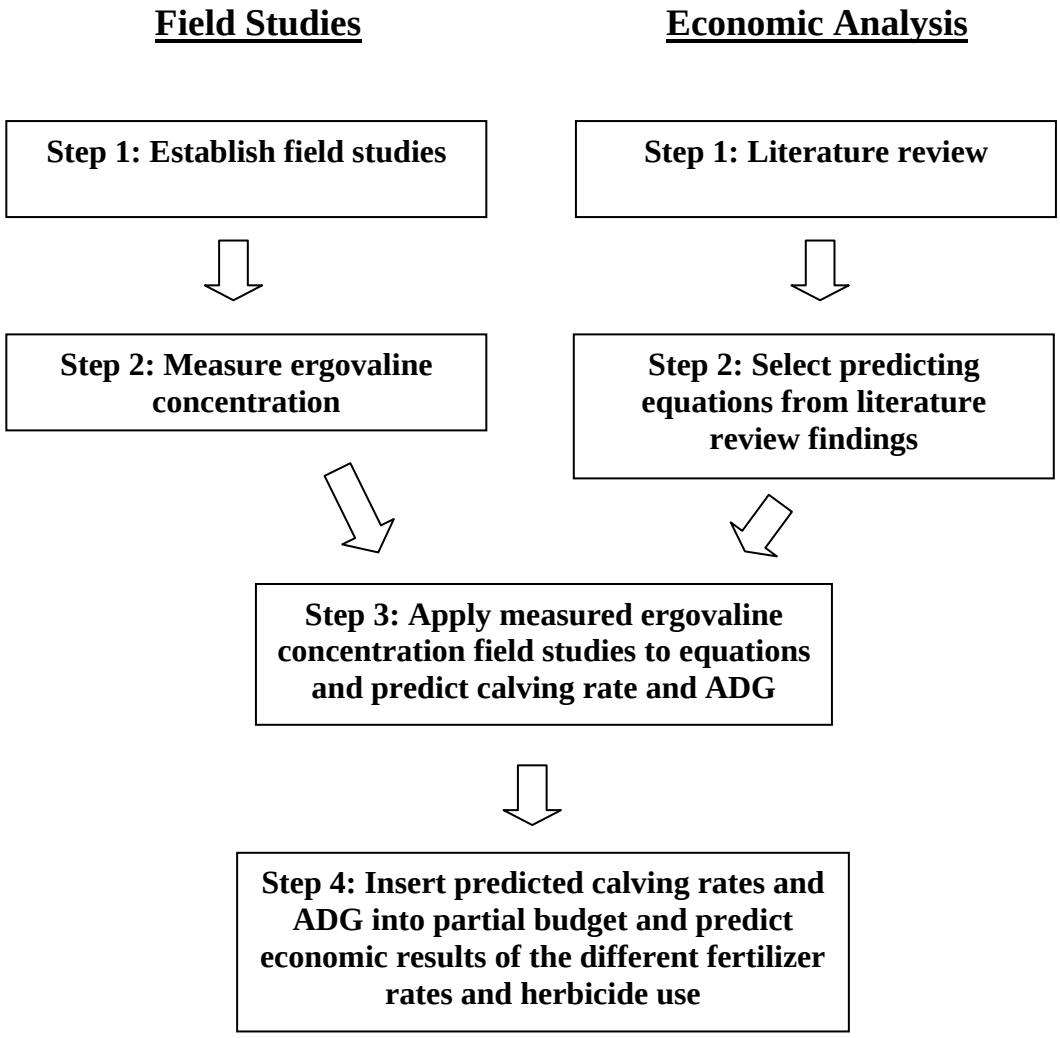

Table 8.1. Literature review of calving rate of cows grazing toxic tall fescue.

Literature	Year	What	What Was Measured	%
Boling, J.A. (Prof. Anim. Sci.)	1985	Calving rate in cows	low endophyte	86
			high endophyte	67
Schmidt et al. (Highlights Agric. Res.)	1986	Conception rate in heifers	low endophyte (0-5%)	96
			high endophyte (80-90%)	55
		Rebred in primiparous cows	low endophyte (0-5%)	93
			high endophyte (80-90%)	33
Beers and Pipers. (AR Farm Res.)	1987	Calving rate in 2-y heifers	Noninfected	90
			endophyte-infected	80
Gay et al. (Appl. Agric. Res.)	1988	Calving rate in cows	Noninfected	94.6
			endophyte-infected	55.4
Wasburn and Green. (Proc. 40th Annu. Conf. N.C. Cattleman's Assoc.)	1991	Calves raised	low endophyte	65
			high endophyte	33
Burke et al. (Theriogenology)	2001	Calving rate	Noninfected	85
			endophyte-infected	85.1
Watson et al. (J. Anim. Sci.)	2004	Calving rate in supplemented cows	AR542 endphyte strain	94
			endophyte-infected (70%)	94
Drewnodki et al. (Livest. Sci.)	2009	Pregnacy rate in heifers	novel endophyte	65
			endophtye-free	65
			endophtye-infected	54

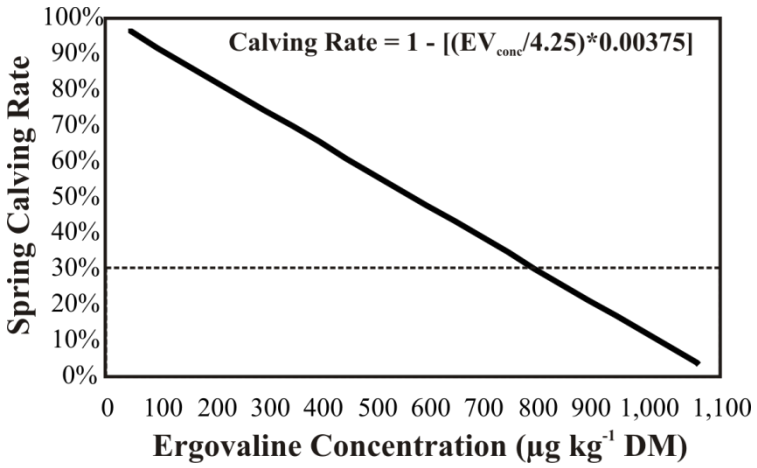

Figure 8.2. Predicted relationship between exposure to ergovaline concentration and spring calving rate as presented in the linear equation used in this analysis. The dashed line represents the calving rate when cows (it is theorized) self-limit ergovaline consumption.

Table 8.2. List of other costs, excluding pasture cost and hay, as used in Missouri Beef, Cow-Calf Enterprise with Spring Calving (FMB7201) projected budget that were not utilized in this analysis' partial budgets for a 50-head beef herd with 42 cows on 200 ac.

Other Costs Per Cow	**Price ($) cow^{-1}**	**Price ($) ac^{-1}**
Protein and Mineral	45.63	9.58
Labor	55.00	11.28
Veterinary, Drugs, and Supplies	25.00	5.25
Utilities & Machinery	148.10	31.10
Facility and Equipment Repairs	8.00	1.68
Breeding Charge		0.00
Capital Replacement (15% of heifer calves)	156.58	32.88
Annual Bull Cost or A.I. Charge	14.50	3.05
Interest on Breeding Stock	92.36	19.40
Insurance on Breeding Stock	10.87	2.28
Professional fees (legal, accounting, etc.)	1.00	0.21
Miscellaneous	6.00	1.26
Depreciation on Facilities and Equipment	10.60	2.23
Interest on Facilities and Equipment	13.87	2.91
Insurance and Taxes on Capital Items	12.19	2.56
Interest on 1/2 Operating Costs @ 9%	18.84	3.96
Total Other Costs	618.52	129.89

Table 8.3. Nutrient, clethodim and clipping input costs used in partial budgets†.

Experiment	Nutrient		Cost of Nutrient	Cost of Nutrient	Application Cost	Total Cost
		(lb ac^{-1})	($ lb^{-1})	($ ac^{-1})	($ ac^{-1})	($ ac^{-1})
Poultry Litter and Chemical NPK Experiment						
Poultry Litter						
	N	78				
1 t	P	41				40.00
	K	67				
Chemical NPK Equivalent						
	N	78	0.45	35.10		
1 t	P	41	0.29	11.89	4.63	81.58
	K	67	0.45	30.15		
Phosphorus Experiment						
Low		50		14.50		19.13
Middle	P	100	0.29	29.00	4.63	33.63
High		200		58.00		62.63
				Cost of Clethodim		
				($ ac^{-1})		
Clethodim Experiment				3.00	5.50	8.50
				Cost of Clipping	**No. Passes**	
				($ ac^{-1})		
Clipping Experiment				$13.00	7	91.00

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

Table 8.4. In addition to other costs† listed in Table 8.3, treatment cost ac^{-1} and cow^{-1} based on the result of varying ergovaline concentration in 'Kentucky-31' tall fescue forage for the nutrient, herbicide and clipping experiments are listed below.

Experiment	($ ac^{-1})	($ cow^{-1})
Poultry Litter and Chemical NPK Equivalent		
Poultry Litter		
2 t ac^{-1}	80.00	380.95
4 t ac^{-1}	160.00	761.90
8 t ac^{-1}	320.00	1,523.81
Chemical NPK Equivalent		
2 t ac^{-1}	158.91	756.71
4 t ac^{-1}	313.19	1,491.38
8 t ac^{-1}	621.75	2,960.71
Phosphorus Experiment		
Low	19.13	91.10
Middle	33.63	160.14
High	62.63	298.24
Clethodim Experiment		
Treated 2005	8.50	40.48
Treated 2006	8.50	40.48
Clipping Experiment	91.00	433.33

†Additional costs are listed in Table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

Table 8.5. Predicted net return ac^{-1} and cow^{-1} based on ergovaline concentration in tall fescue forage measured in each of the analyzed experiments. Other costs (Table 3) are not considered.

Experiment	Calving Rate		
	-----(%)-----	($ ac^{-1})	($ cow^{-1})
Poultry Litter And Chemical NPK Equivalent Experiment			
Non-treated Control	63	95.15	453.10
Poultry Litter			
2 t ac^{-1}	69	22.51	107.20
4 t ac^{-1}	57	-72.21	-343.83
8 t ac^{-1}	62	-226.07	-1,076.54
Chemical NPK Equivalent			
2 t ac^{-1}	62	-64.85	-308.85
4 t ac^{-1}	56	-266.62	-1,069.62
8 t ac^{-1}	55	-536.41	-2,554.33
Phosphorus Experiment			
Non-treated Control	52	81.66	388.87
Low	60	72.34	344.50
Middle	52	52.66	250.77
High	50	21.21	101.00
Clethodim Experiment			
Non-treated 2005	57	87.79	418.07
Treated 2005	73	98.82	471.03
Non-treated 2006	17	38.74	184.46
Treated 2006	77	103.92	494.86
Clipping Experiment			
Non-clipped			
550 µg ergovaline kg^{-1} DM	51	80.44	383.05
Clipped			
325 µg ergovaline kg^{-1} DM	71	13.97	66.50
350 µg ergovaline kg^{-1} DM	69	11.51	54.82
375 µg ergovaline kg^{-1} DM	67	9.06	43.14

Table 8.6. Results of partial budget† for the Poultry Litter‡ and Chemical Equivalent Fertilizer£ experiment. Returns over cost considered.

Treatment	Control	Poultry Litter†	Chemical Equivalent to Poultry Litter‡
		(2 t ac^{-1})	(2 t ac^{-1} equivalent)
Ergovaline Concentration (µg kg^{-1} DM)	425	355	432
Spring Calving Rate (%)	63	69	62
Returns Per Cow Per Year			
Steers	200.97	220.11	197.78
Heifers	176.40	193.20	173.60
Cull Cows	87.36	87.36	87.36
Gross Return ($) Per Cow	464.73	500.67	458.74
Listed Cost Per Cow			
Fertilizer Cost ($)	--	380.95	756.71
Marketing Cost ($)	11.62	12.52	11.47
Sum of Listed Cost Per Cow	11.62	393.47	768.18
Net Return ($) Per Cow	453.11	107.20	-309.44
Net Return ($) Per ac	95.15	22.51	-64.98

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

‡ Cost of poultry litter = \$80 $(2\ t)^{-1}$

£ Cost of chemical fertilizer: N=\$0.45 lb^{-1}, P=\$0.29 lb^{-1}, K=\$0.45 lb^{-1}

Table 8.7. Results of partial budget† for the Phosphorus experiment‡. Returns over cost considered.

Treatment	**Phosphorus† (lb ac^{-1})**			
	0	50	100	200
Ergovaline Concentration (µg kg^{-1} DM)	546	448	544	563
Spring Calving Rate (%)	52	60	52	50
Returns Per Cow Per Year				
Steers	165.88	191.40	165.88	159.50
Heifers	145.60	168.00	145.60	140.00
Cull Cows	87.36	87.36	87.36	87.36
Gross Return Per Cow	398.84	446.76	398.84	386.86
Listed Cost Per Cow				
Fertilizer Cost ($)	--	91.10	138.10	276.19
Marketing Cost ($)	9.97	11.17	9.97	9.67
Sum of Listed Cost Per Cow	9.97	102.27	148.07	285.86
Net Return ($) Per Cow	388.87	344.50	250.77	101.00
Net Return ($) Per ac	81.66	72.34	52.66	21.21

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

‡ Cost of P=$0.29 lb^{-1}

Table 8.8. Results of partial budget† for the Herbicide Clethodim experiment. Returns over cost considered.

	Herbicide			
	2005		2006	
	Control	Treated	Control	Treated
Ergovaline Concentration (µg kg^{-1} DM)	492	301	942	257
Spring Calving Rate (%)	57	73	17	77
Returns Per Cow				
Steers:	181.83	232.87	54.23	245.63
Heifers	159.60	204.40	47.60	215.60
Cull Cows	87.36	87.36	87.36	87.36
Gross Return Per Cow	428.79	524.63	189.19	548.59
Listed Cost Per Cow				
Herbicide Cost ($)	--	40.48	--	40.48
Marketing Cost ($)	10.72	13.12	4.73	13.71
Sum of Listed Cost Per Cow	10.72	53.60	4.73	53.65
Net Returns ($) Per Cow	418.07	471.03	184.46	494.84
Net Returns ($) Per ac	87.79	98.92	38.74	103.92

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

Table 8.9. Results of predicted partial budget† for clipping once a month for 7 months (April to October). Ergovaline concentration of mowed 'Kentucky-31' tall fescue represented range of concentrations. Returns over cost considered.

	Non Clipped	**Clipped**		
Ergovaline Concentration (µg kg^{-1} DM)	550	325	350	375
Spring Calving Rate (%)	51	71	69	67
Returns Per Cow				
Steers	162.69	226.49	220.11	213.73
Heifers	142.80	198.80	193.20	187.60
Cull Cows	87.36	87.36	87.36	87.36
Gross Return Per Cow	392.85	512.65	500.67	488.69
Listed Cost Per Cow				
Season Clipping Cost ($)	--	433.33	433.33	433.33
Marketing Cost ($)	9.82	12.82	12.52	12.22
Sum of Listed Cost Per Cow	9.82	446.15	445.85	445.55
Net Returns ($) Per Cow	383.03	66.50	54.82	43.14
Net Return ($) Per ac	80.44	13.97	11.51	9.06

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

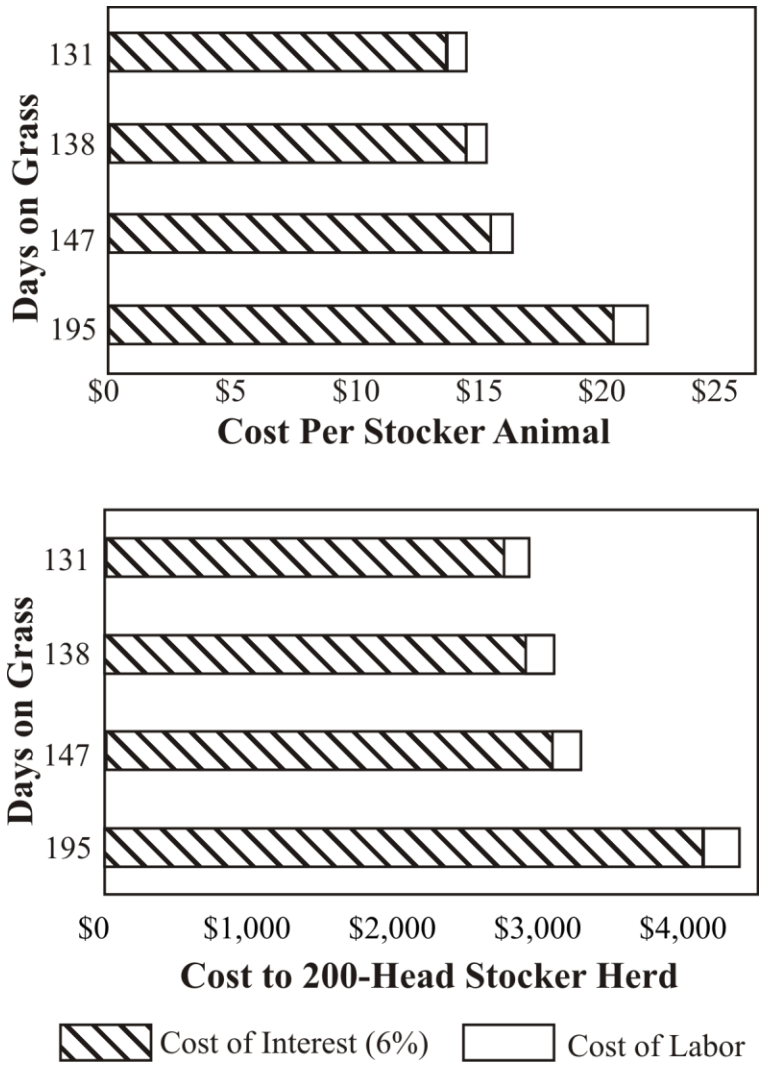

Figure 8.3. Comparison among grazing days of cost of interest (6%) and labor for the time period for stocker animals to reach desired 235 lb gain.

Table 8.10. Result of three nutrient and herbicide treatment partial budgets on stocker steers and heifers.

Treatment	Ergovaline Concentration	ADG (lb)		Days on Grass	Hundred Weight (lb) Produced		Combined Cost of Labor and Loan Interest (6%)	
	(µg kg^{-1} DM)	Steer	Heifer		Steer	Heifer	(ac^{-1})	($animal^{-1}$)
Poultry Litter and Chemical NPK Equivalent								
Non-treated Control	425	1.7	1.5	138	2.35	2.11	15.43	15.43
Poultry Litter								
2 t ac^{-1}	355	1.7	1.5	138			15.43	15.43
4 t ac^{-1}	493	1.6	1.4	147	2.35	2.11	16.43	16.43
8 t ac^{-1}	436	1.6	1.4	147				
Chemical NPK Equivalent								
2 t ac^{-1}	432							
4 t ac^{-1}	496	1.6	1.4	147	2.35	2.11	16.43	16.43
8 t ac^{-1}	511							
Phosphorus Experiment								
Non-treated Control	546							
Low	448	1.6	1.4	147	2.35	2.11	16.43	16.43
Mid	544							
High	563							
Clethodim Experiment								
Non-Treated 2005	492	1.6	1.4	147			16.43	16.43
Non-Treated 2006	942	1.2	1.0	195	2.35	2.11	21.80	21.80
Treated 2005	301	1.8	1.6	131			14.65	14.65
Treated 2006	257	1.8	1.6	131				

†Additional costs are listed in table 8.3. Depending on the cattle cycle and input cost, actual net values may be negative when these additional costs are included. These partial budgets are considered such that specific attention can be given to the estimated consequences of the tests (herbicide use, fertilizer rate, or clipping) under consideration.

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Chapter 9: Dissertation Summary

Effective management of tall fescue forage production can affect ergovaline and total ergot alkaloid concentrations. Managing forage production, and by extension ergovaline and total ergot alkaloid concentrations, can also improve animal performance.

In this dissertation, five management practices were studied. These included various fertilizer rates, fertilizer forms, herbicide application, and monthly clipping. These practices were tested to determine if they could reduce ergovaline and total ergot alkaloid concentrations, and if so, were cost-effective practices. Of these five practices, results of four experiments--herbicide use, poultry litter fertilizer and its chemical NPK equivalent, P fertilizer and monthly clipping—were subjected to economic analysis.

In the herbicide experiment discussed in Chapter 2, clethodim reduced ergovaline and total ergot alkaloid concentrations in both years, 2005 and 2006. While there was a year x treatment interaction for both forage ergovaline and total ergot alkaloid concentration, clethodim had a greater impact on alkaloids than did growing conditions. The effect of clethodim on alkaloid concentrations can be explained by its suppression of seedheads, where ergovaline and ergot alkaloids are highly concentrated.

As discussed in Chapter 3, poultry litter fertilizer and its chemical NPK equivalent were studied for their effects on ergovaline and total ergot alkaloid concentrations. In the first year of the experiment, chemical-treated tall fescue forage contained more ergovaline than litter-treated tall fescue. Also in the first year, tall fescue receiving high rates of fertilizer contained more ergovaline than forage receiving low rates of fertilizer and the non-treated control. There was no effect in the second and third

years, indicating a lack of nutrient carry-over effect after the year of application. There was no significant main effect or interaction of treatments on total ergot alkaloid concentration.

In the P fertilizer experiment, as discussed in Chapter 4, there was no treatment effect of a one-time fertilizer application on ergovaline or total ergot alkaloid concentration when tall fescue was fertilized with three rates of P. However, there was a year effect. Between 2004 and 2006, ergovaline concentration decreased from 713 µg ergovaline kg^{-1} DM in 2004, to 513 µg ergovaline kg^{-1} DM in 2005 to 350 µg ergovaline kg^{-1} DM in 2006. There was also a year effect on total ergot alkaloid concentration; concentration was 715 µg total ergot alkaloid kg^{-1} DM in 2004, 644 µg total ergot alkaloid kg^{-1} DM in 2005 and 720 µg total ergot alkaloid kg^{-1} DM. For two reasons this experiment should be repeated. First, the treatment effect approached significance, at $\alpha = 0.15$. Second, even after treatment, the soil P concentration was well below the soil P levels recommended by the University of Missouri Extension for tall fescue forage production.

As described in Chapter 5, limestone form and rate of application had no effect on forage ergovaline or total ergot alkaloid concentrations. However, this experiment used a 'Kentucky-31' tall fescue site with a 30% endophyte infection rate. The effect of limestone on forage ergovaline and total ergot alkaloid concentration may be observable when endophyte-infection is greater than 90%. This experiment should be repeated on a site with a high percentage of endophyte-infected tall fescue.

As discussed in Chapter 6, ergovaline and total ergot alkaloid concentration in monthly regrowth of 'Kentucky-31' tall fescue (April through October) was similar among three locations: Georgia, Missouri and South Carolina. Ergovaline concentration was <300 µg ergovaline kg^{-1} DM in April and increased over the seven month period, peaking to >800 µg ergovaline kg^{-1} DM in October. While there was a month x location interaction, ergovaline concentrations reflected a season effect more than a location effect.

There was a month x location interaction for total ergot alkaloid concentration in monthly regrowth, as total ergot alkaloid concentration was not similar during the months among the locations of Georgia, Missouri and South Carolina. However, there was a general pattern that occurred in the form of a bimodal curve. Total ergot alkaloid concentration first peaked during April and May in Georgia and South Carolina and during June and July in Missouri. Concentration peaked a second time for all three locations during October. Because the seedheads were removed by the monthly clipping, the total ergot alkaloid peaks could not have been related to *Claviceps*, the surface pathogen that infects the seedheads. The bimodal peaks did correspond to the two times during the growing season, spring and early autumn, when tall fescue and its endophyte experience rapid growth.

As discussed in Chapter 7, month affected ergovaline and total ergot alkaloid concentrations in non-clipped tall fescue that was allowed to accumulate over the calendar year (January through December). Ergovaline concentration was represented by a bimodal curve that was not similar among Georgia, Missouri and South Carolina. Local

growing conditions influenced particularly the start of the growing season, when growth, and by extension increasing ergovaline concentrations, were initiated. The first peak of the bimodal curve, which occurred in late spring and early summer, likely represented the development and maturing of seedheads. The second peak of the bimodal curve represented a surge of forage growth in autumn with the return of cooler and wetter weather.

In the economic analysis described in Chapter 8, the simulated, economic impact of ergovaline concentration on calving rate and stocker gains was observed in net returns associated with clethodim, various fertilizers, and monthly clipping management practices. Among all management practices studied in this dissertation, the herbicide effect was most economically favorable. The highest calving rate, 77%, and the largest net return, \$103.92 ac^{-1}, were simulated in the clethodim-treated tall fescue.

The simulated effect of poultry litter fertilizer and its chemical NPK equivalent predicted higher calving rates with poultry litter use than with chemical fertilizer. Calving rate was predicted to be 67% for the 2 t poultry litter ac^{-1}. Calving rates were predicted to range from 55% to 62% for the chemical NPK equivalent fertilizer. While higher calving rates can increase gross return per cow or per acre, once the cost of fertilizer was calculated, gains due to higher calving rate were eroded. The predicted net returns for forage treated with chemical fertilizer were all negative values. The only fertilizer treatment among rates of poultry litter and chemical NPK equivalent, 2 t poultry litter ac^{-1}, was predicted to produce forage with a positive net return, \$22.51 ac^{-1}.

Phosphorus treatment produced ergovaline concentrations that in turn were used to predict calving rates between 50% and 60%. The P treatment that predicted the highest net return from calving rate from this experiment, \$81.66 ac^{-1}, was from the non-treated control. Phosphorus treatment and the non-treated control did not differ in predicted stocker ADG, 1.6 lb d^{-1}, or in net return ac^{-1}, \$16.43.

The effect of monthly clipping on predicted calving rate varied with the range of ergovaline concentrations. Based on forage ergovaline concentration, the effect of monthly clipping predicted calving rates that ranged from 67% to 71% and net returns ranging from \$9.06 $ac^{-1}$to \$13.06 ac^{-1}. Returns were lowered by the monthly cost of clipping, which is probably not a feasible practice for many reasons. It was not possible in this analysis to test the effect of a single clipping event on ergovaline and subsequent effects on calving rate. Such a practice, single clipping, may prove economically feasible. It would avoid the recurring cost of monthly clipping and at the same time would reduced the toxin in the field and thereby increase the caliving rate.

In the case of tall fescue allowed to accumulate over the calendar year, the corresponding ergovaline resulted in a predicted calving rate for the non-clipped forage of 51% and a net return of \$80.44 ac^{-1}.

Conclusions

Clethodim and nutrient management can reduce ergovaline and increase the herd calving rate and stocker gains in animals exposed to ‘Kentucky-31’ tall fescue. In addition, monthly clipping management can reduce ergovaline concentrations early in the growing season because ergovaline-rich seedheads are removed.

Of all management practices studied, clethodim was the most effective and cost-effective, as it lowered ergovaline concentration but did not require high input costs.

VITA

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