FUNDED GRANT COMPLETION REPORT

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A BASLINE SURVEY OF ENDOPHYTE INFECTION OF FESCUE PASTURES IN GEORGIA

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A BASLINE SURVEY OF ENDOPHYTE INFECTION OF FESCUE PASTURES IN GEORGIA

Introduction

Tall Fescue (*Lolium arundinaceum*), a cool season perennial grass, grows very well in north Georgia. With over a million acres of pasture, tall fescue has been an important forage base for beef cattle in north Georgia. Though a substantial proportion of the total yearly production of fescue occurs in spring, this grass is also productive during early summer, fall, and late winter as well as in mid-summer if moisture conditions are favorable. Furthermore, stockpiling this grass in pastures and hay fields during late summer to early fall allows grazing during late fall to early winter, which can considerably reduce the fall and winter feed costs.

The fungus *Neotyphodium coenophialum* grows inside the fescue plant (thus called "endophyte") and is transmitted via seed. The endophyte confers several benefits to fescue pasture sustainability such as drought and stress tolerance (West et al., 1993), resistance to herbivorous insects (Rowan and Latch, 1994; Shymanovich et al., 2015), as well as resistance to pathogenic fungi, viruses and root-feeding nematodes (Latch, 1997), and persistence by preventing over-grazing. The fungus also benefits from this relationship because the plant provides energy and a sustainable environment for the fungus (Adcock et al., 1997). This beneficial symbiotic relationship between the plant and fungus is considered necessary to ensure optimal plant production and survival (Adcock et al., 1997; Alan and Stuedemann, 2006).

However, the endophyte produces toxins called ergot-alkaloids, including a group of compounds related to lysergic acid (Shelby and Kelley, 1991). The toxic alkaloids cause "fescue toxicosis" and exert toxic effects in livestock that graze infected pastures or consume hays from infected field (Stuedemann et al., 1998) which cost beef cattle farmers a billion dollars annually in lost revenue each year in the United States alone (Bouton, 2007; Waller, 2009). Various adverse effects of fescue toxicosis on animals have been reported, including "summer syndrome", "fescue foot", reproductive difficulties, reduced weight gain, decreased milk production, slightly elevated body temperature, impaired heat tolerance, excessive nervousness, and failure to shed winter hair coats in the spring (Craig et al., 1994).

Historically, a vast majority of tall fescue pastures in Georgia and other places in the USA are infected by toxin producing endophyte. Infection level can be highly variable among the pastures or even within a single fescue pasture (Stuedemann and Hoveland, 1988). In pastures with less than 20-35% endophyte infection, the toxins can be diluted by inter-seeding legumes (usually white clover or red clover) or planting other grasses (e.g., bermudagrass, orchardgrass, etc.) (Roberts and Andrae, 2004; NRCS, 2011). However, there is a lack of clear and precise information about the nature and extent of endophyte infection in Georgia fescue pastures.

It is not possible at present to "cure" fescue infected with toxic endophytes. Mitigation of the problem requires renovation of heavily-infected pasture with fescue not infected by the toxic endophyte. Endophyte-free varieties have been tried in Georgia since the 1990s, but research has shown that they fail to persist well under adverse growing conditions (Franzluebbers and Stuedemann, 2005). As a result, these fescue varieties are not generally recommended in Georgia.

In contrast, newer novel-endophyte fescue varieties do not produce toxic ergot alkaloids, but possess the positive agronomic characteristics of a toxin-producing endophyte (Hopkins et al., 2010). Renovation with tall fescue varieties that are infected with a novel endophyte is considered as the best option for pastures that have endophyte infection 60% or greater (NRCS, 2011). Although replacement of an existing fescue pasture with novel-endophyte infected tall fescue is a costly investment initially, the economists from UGA have found that this replacement is a cost-effective strategy for nearly all forage-based livestock systems in Georgia when severity of toxic endophyte infection demands such renovation (Hancock et al., 2018).

However, the extent and severity of toxic endophyte infection in fescue pastures and hay fields in Georgia is largely unknown. Hence, we lack information on the adverse economic consequences of fescue toxicosis in the beef cattle industry necessary to design and implement appropriate strategies to combat this important problem. To address this lack of information, we proposed to conduct a survey to assess the severity of toxic endophyte infection in fescue pastures critical to the beef cattle industry in Georgia.

This baseline survey was conducted with a grant funding from Georgia Commodity Commission for Beef Cattle to have a preliminary assessment of the extent and severity of toxic endophyte infection in the one million acres of fescue pastures in Georgia. The over-arching goal of this research was to collect, analyze, and summarize analytical results from samples of tillers for endophyte infection collected from fescue pasture in north Georgia. This information should provide valuable insight into existing fescue pasture management decisions, and the extent and urgency of the need for remedial action, such as pasture renovation. The ultimate objective was to develop a "risk profile" for the fescue industry by estimating the percentage of acreage in different risk categories. This would guide educational and remedial programs to target the most needed areas for mitigation.

Materials and Methods

This research was conducted using a survey approach to assess the status of endophyte infection in the fescue pastures in Georgia. Samples were collected through collaborative efforts of the grant team and the ANR Extension Agents of various counties. Emphasis was given to have samples collected as broadly as possible across the fescue belt from approximately Eatonton north.

In addition to testing individual pastures for endophyte infection, we also conducted a smaller-scale study of spatial variability in infection within a field by taking 4 samples from each of the 2 different fescue pastures (F1 and F2) in the J. Phil Campbell Sr. Research and Education Center Farm of the University of Georgia, located in Watkinsville, Georgia. For this each of the 2 pastures was divided into 4 quadrants primarily based on topography and one sample (30-40 tillers) was collected from each quadrant. This was done to assess if there was a need for taking multiple samples from a single pasture and for interpreting the results properly.

The Extension forage specialists worked with local Extension agents to identify pasture and hay fields representative of their respective counties. As the goal of this project was to evaluate the nature and extent of toxic endophyte infection, all necessary measures were taken to exclude samples from the pastures that are known to be novel-endophyte varieties.

The project team developed a special sample submission form (**see Appendix I**) and an easy-to follow step-by-step sampling protocol (**see Appendix II**). Furthermore, the team also developed a video instruction in collaboration with UGA Professor Dr. Nicholas Hill (a renowned fescue endophyte specialist) and posted it on YouTube (https://youtu.be/s6eF4XBbv7g). A link of this YouTube video was also included in the sampling protocol to ensure all samplers uniformly follow the recommended protocol and the samples adequately represent the fescue pastures.

The sampling kits with various materials to be used for sample collection and submission were sent to the cooperating Extension agents. A sampling kit (**Appendix Figure 1**) had the following components:

1. A printed copy of the step-by-step sampling protocol
2. A printed copy of submission form
3. An Insulated Foam Shipping Kit - 6" x 4.5" x 3"
4. A Cold Pack (Ice Brick): 5.5" x 4" x 0.75"
5. A quart size Zip-lock bag with a submission form in it
6. A gallon size Zip-lock bag with a piece of paper towel in it, to be used for sample
7. A Prepaid UPS shipping label to Send the Sample to The Feed and Environmental Laboratory

We received and analyzed a total of 56 samples (each containing 30-40 tillers) from 21 Georgia counties. Except Oconee county, each sample represented an individual pasture. The 8 samples from Oconee county were from 2 pastures, 4 from each as described earlier. Thus, the study surveyed 50 pastures (56 - 8 +2 = 50). Table 1 below includes the list of samples with cooperating county Extension agents.

Table 1: List of 56 samples received and analyzed.

Date	Lab. ID#	Sample ID	Agent	County
7/1/2021	11918	Rylee 2	Zach McCann	Banks
	11919	Rylee 1	Zach McCann	
	11920	Bregenzer	Zach McCann	
7/7/2021	114	Wade 1689	Paul Pugliese	Bartow
	115	Bartow 1910	Paul Pugliese	
10/1/2021	3287	NP	Paula Burke/Angie Stober	Carroll
	3288	CP	Paula Burke/Angie Stober	
7/8/2021	155	NO ID	Josh Fuder/John Bennett	Cherokee
10/18/2021	4024	Hamby	Clark MacAllister/Jason Hamby	Dawson
11/11/2021	4890	Pasture 1	Ashley Hoppers/Kenny Holland	Fannin
12/2/2021	5414	Lory Hay	Ashley Hoppers/Jake Williams	
12/14/2021	5773	NO ID	Ashley Hoppers/Seth Davis	
11/30/2021	5339	Heifer Pasture	Keith Mickler/Kimani Grey-Campbell	Floyd
7/28/2021	742	1	Raymond Fitzpatrick	Franklin
8/6/2021	1219	1	Raymond Fitzpatrick	

Table 1. List of 56 samples received and analyzed (Continued).

Date	Lab. ID#	Sample ID	Agent	County
10/18/2021	4025	Eli Gilmer Pasture	Garrett Hibbs	Hall
	4026	Phil Benely Pasture	Garrett Hibbs	
11/17/2021	4972	Tommy Blackstock Sample	Garrett Hibbs	
6/17/2021	11406	Brown 334	Greg Pittman	Jackson
	11407	Greg Pittman	Greg Pittman	
	11408	Bell	Greg Pittman	
7/6/2021	56	PK	Lucy Ray/Tatumn Behrens	Morgan
	57	NH	Lucy Ray/Tatumn Behrens	
12/2/2021	5474	Ainslie	Lucy Ray/Tatumn Behrens	
6/24/2021	11787	DW Sewell	Ashley Best	Newton
	11788	DW Knox Farm	Ashley Best	
	11789	DW Jamestown	Ashley Best	
8/3/2021	1022	F1Q1 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	Oconee
	1023	F1Q2 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1024	F1Q3 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1025	F1Q4 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1026	F2Q1 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1027	F2Q2 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1028	F2Q3 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
	1029	F2Q4 - JPC Farm	Saha, Ronaghi, Parks, FEW Lab	
6/15/2021	11289	Kitchens Farms	Mary Carol Sheffield	Paulding
	11290	Doug Sowar	Mary Carol Sheffield	
7/13/2021	294	Site 1	Brooklyne Wassel	Pike
	295	Sample 2	Brooklyne Wassel	
6/9/2021	10840	Tradut Farm	Thad Glenn	Stephens
	10841	School Farm	Thad Glenn	
	10842	Ag Center Hayfield	Thad Glenn	

Table 1. List of 56 samples received and analyzed (Continued).

Date	Lab. ID#	Sample ID	Agent	County
6/22/2021	11636	Ferrell Farm	Deborah Xavier_Mis	Troup
7/13/2021	296	Allen Farm	Deborah Xavier_Mis	Troup
10/8/2021	3481	Owenby	Jacob Williams	Union
	3482	Hutson	Jacob Williams	Union
	3483	Nealy	Jacob Williams	Union
12/8/2021	5666	Bradley	Jacob Williams	Union
11/5/2021	4662	Feed Barn	Hailey Robinson/Wes Smith	Upson
	4663	Dry Lot Powerline	Hailey Robinson/Wes Smith	
	4664	Powerline	Hailey Robinson/Wes Smith	
12/3/2021	5529	Hart	Wade Hutcheson	Walker
7/21/2021	559	Henderson	Roger Gates	Whitfield
12/15/2021	5890	Church Pasture	Roger Gates	
6/10/2021	10960	Bobby King	Roger Gates	
6/16/2021	11288	Bethel	Roger Gates	

Endophyte analysis in the tiller samples was conducted using proprietary "Phytoscreen Neotyphodium immunoblot detection kit (Agrinostics Ltd. Co, Watkinsville, GA, agrinostics.com)". All laboratory analyses were conducted by staff of the University of Georgia Agricultural and Environmental Services Laboratory, Athens, GA (aesl.ces.uga.edu).

Results and Discussion

As depicted in Table 2, all 50 fescue pastures sampled from 21 counties turned out to be severely infected by endophyte. Except for the one sample from Walker county which had 70% infected tillers and 3 from Upson county that had a mean infection level of 73%, the mean infection levels in the rest of the samples from 19 counties varied from 87 to 100%. The lowest infection of 25% was observed in a pasture from Upson county, but the other two pastures from this county had 95 and 100% infection. The mean infection level for the whole study was 94% with a median of 100%. Out of 56 samples tested, 30 had 100% infected tillers. Thus, we found endophyte infection in the fescue pastures in Georgia is widespread and mostly severe.

In addition to testing one sample from per pasture, we also tested 4 samples from each of the two pastures (F1 and F2) by dividing them into 4 quadrants (Q1, Q2, Q3, and Q4) primarily considering the topographical variation within the pastures. This was a smaller-scale study of spatial variability in infection within two pastures in the J. Phil Campbell Sr. Research and Education Center Farm of the University of Georgia, located in

Watkinsville, Oconee County. However, the results show very minimal intra-pasture variability in endophyte infection with the levels of infection ranging from 90-100% in one pasture (F2) and 95-100% in another (F1).

Table 2. Extent of endophyte infection in the 50 fescue pastures sampled in 21 Georgia counties.

County	No. of Tillers Tested	No. of Tillers Infected	% Tillers infected	Sample counts
Banks	20	20	100	
Banks	20	20	100	
Banks	20	20	100	
		Mean:	**100.0**	**3**
Bartow	20	19	95	
Bartow	20	19	95	
		Mean:	**95.0**	**2**
Carroll	20	20	100	
Carroll	20	20	100	
		Mean:	**100.0**	**2**
Cherokee	25	23	92	
		Mean:	**92.0**	**1**
Dawson	20	20	100	
		Mean:	**100**	**1**
Fannin	20	20	100	
Fannin	20	18	90	
Fannin	20	17	85	
		Mean:	**91.7**	**3**
Floyd	24	21	87.5	
		Mean:	**87.5**	**1**
Franklin	20	20	100	
Franklin	20	18	90	
		Mean:	**95**	**2**

Table 2. Extent of endophyte infection in the 50 fescue pastures sampled in 21 Georgia counties (continued).

County	No. of Tillers Tested	No. of Tillers Infected	% Tillers infected	Sample counts
Hall	20	20	100	
Hall	20	19	95	
Hall	20	20	100	
		Mean:	**98.3**	**3**
Jackson	20	19	95	
Jackson	20	17	85	
Jackson	20	18	90	
		Mean:	**90.0**	**3**
Morgan	28	28	100	
Morgan	26	22	84.62	
Morgan	20	20	100	
		Mean:	**94.9**	**3**
Newton	20	20	100	
Newton	23	23	100	
Newton	20	20	100	
		Mean:	**100.0**	**3**
Oconee	20	20	100	
Oconee	20	19	95	
Oconee	20	20	100	
Oconee	20	20	100	
Oconee	20	18	90	
Oconee	20	19	95	
Oconee	20	19	95	
Oconee	20	20	100	
		Mean:	**96.9**	**8**
Paulding	20	19	95	
Paulding	20	19	95	
		Mean:	**95.0**	**2**

Table 2. Extent of endophyte infection in the 50 fescue pastures sampled in 21 Georgia counties (continued).

County	No. of Tillers Tested	No. of Tillers Infected	% Tillers infected	Sample counts
Pike	20	20	100	
Pike	20	19	95	
		Mean:	**97.5**	**2**
Stephens	20	20	100	
Stephens	20	18	90	
Stephens	20	20	100	
		Mean:	**96.7**	**3**
Troup	20	20	100	
Troup	20	20	100	
		Mean:	**100**	**2**
Union	20	20	100	
Union	20	16	80	
Union	20	19	95	
Union	20	18	90	
		Mean:	**91.3**	**4**
Upson	20	19	95	
Upson	20	20	100	
Upson	20	5	25	
		Mean:	**73.3**	**3**
Walker	20	14	70	
		Mean:	**70.0**	**1**

Table 2. Extent of endophyte infection in the 50 fescue pastures sampled in 21 Georgia counties (continued).

County	No. of Tillers Tested	No. of Tillers Infected	% Tillers infected	Sample counts
Whitfield	21	21	100	
Whitfield	20	20	100	
Whitfield	20	20	100	
Whitfield	20	20	100	
		Mean:	**100.0**	**4**
		Overall:		**56**
		MIN	**25.0**	
		MAX	**100.0**	
		Mean	**94.4**	
		Median	**100.0**	
		Mode	**100.0**	
		SD	**11.3**	

A color coded map using the mean endophyte infection levels observed in the 21 counties is presented in Figure 1.

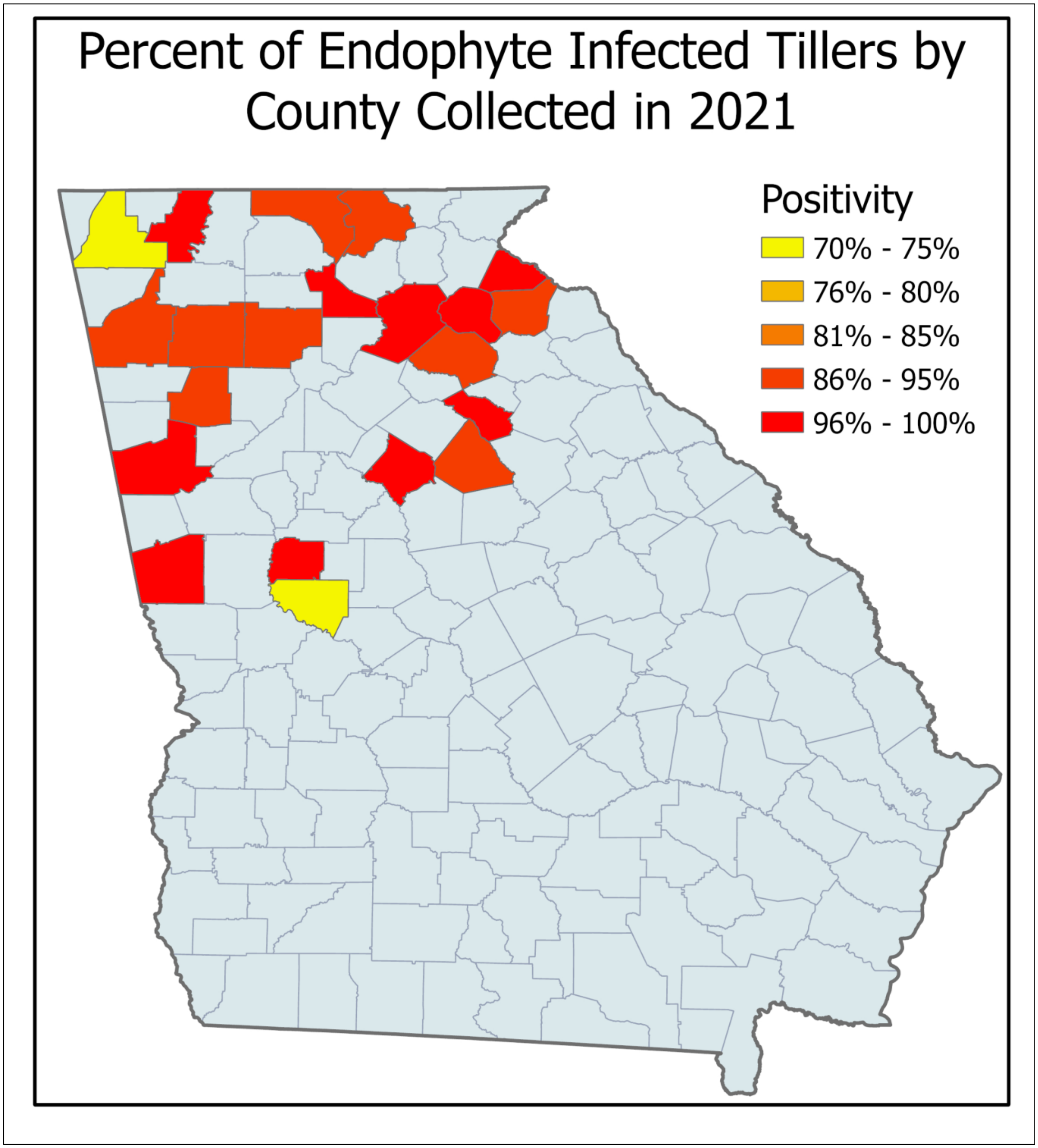

Figure 1. A map showing the severity of endophyte infection in fescue pastures in various Georgia counties observed through this study.

Implications of the Study Results

According to recommendations from the NRCS, only in pastures with less than 20-35% endophyte infection, the toxins can be diluted by inter-seeding legumes (usually white clover or red clover) or planting other grasses (e.g., bermudagrass, orchardgrass, etc.) (NRCS, 2011). However, only one pasture (from Upson county) fell within this range with a 25% endophyte infection would qualify for applying this recommendation.

According to NRCS (2011), renovation with tall fescue varieties that are infected with a novel-endophyte that do not produce toxic ergot alkaloids while still providing positive agronomic advantages of a toxin-producing endophyte, is the best option for pastures that have 60% or greater endophyte infection. Thus, all other 49 pastures (except the one from Upson county) of this study, with 70-100% endophyte infection, merit renovation with newer novel-endophyte fescue varieties. This replacement would be a costly investment initially, but this is going to be a cost effective intervention for the fescue-based livestock systems in the long run as found by the economists from UGA (Hancock et al., 2018).

We also believe that this baseline survey results will serve as a teaching tool to convince farmers to have their own pastures tested in order to assess the need for remedial management. Since NRCS offers cost-assistance for renovation of fescue pastures with novel endophyte varieties of fescue in some states within the fescue belt, including Georgia, the impact of this survey results are much higher. Furthermore, the outcomes of this survey will help with the current work of the Alliance for Grassland Renewal (https://grasslandrenewal.org).

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APPENDICES

Appendix Figure 1: Different components of a fescue endophyte sampling kit used for the study.

Appendix I: Fescue endophyte sample submission form used for the study

The University of Georgia

Feed and Forage Research Group
Feed and Environmental Water Laboratory, 2300 College Station Road, Athens, GA 30602
Tel: 706-542-5350/7690; Fax: 706-542-1474

Fescue Endophyte Sample Submission Form

Project Name: A Baseline Survey of Endophyte Infection of Fescue Pastures in Georgia
Main Implementing Department: Agricultural and Environmental Services Laboratories
Investigators:
Dr. Uttam Saha (sahau@uga.edu); Dr. Jason Lessl (jlessl@uga.edu); Daniel Jackson (djackso@uga.edu)
Dr. Lisa Baxter (baxterl@uga.edu); Dr. Lawton Stewart (lawtons@uga.edu)
Dr. Jennifer Tucker (jjtucker@uga.edu); Philip Brown (philip.brown2@usda.gov)

County:______________________________

Sampler:_____________________________ _ Date of Sampling: __________________

Agent:_______________________________

Contact Details

Address:_____________________________ _ E-mail: ________________________ _

City:_________________________________

State: _____ Zip: ___________ Phone: __________________

SAMPLE INFORMATION	
Following the TIPS provided in the "Sampling Protocol", have you confirmed the selected sampling unit is a FESCUE pasture/field: ☐ **YES** ☐ **NO**	
Pasture/Field Address:	Poultry Litter Applied (this year or previous year): ☐ YES ☐ NO Estimated Rate (lb/ac):
City or Town:	
Zip:	Is the fescue pasture also planted with other forage species (like clover)? ☐ YES ☐ NO If YES, name(s) of the other forage species: 1. 2. 3. 4. Approximate coverage of all other forage species (combined): %
GPS reading (if possible):	
Soil Type (e.g., Sandy/Loamy/Silty/Clayey, etc.):	
Number of Tillers in the Sample (**30 required**):	

Appendix II: Fescue endophyte sample submission and submission protocol used for the study

The University of Georgia

Feed and Forage Research Group
Feed and Environmental Water Laboratory, Agricultural and Environmental Services Laboratories
2300 College Station Road, Athens, GA 30602
Tel: 706-542-5350/7690; Fax: 706-542-1474

Project Name: A Baseline Survey of Endophyte Infection of Fescue Pastures in Georgia
Main Implementing Department: Agricultural and Environmental Services Laboratories
Investigators: Dr. Uttam Saha, Dr. Jason Lessl, Daniel Jackson, Dr. Lisa Baxter, Dr. Lawton Stewart, Dr. Jennifer Tucker, and Philip Brown
Advisor: Dr. Nicholas Hill
Contact Phone Number (for questions related to sampling and submission):

- Dr. Lisa Baxter: 229-472-0160 (cell)
- Dr. Uttam Saha: 706-461-9489 (cell)

FESCUE ENDOPHYE SAMPLE COLLECTION AND SUBMISSION PROTOCOL

When to Sample

Endophyte sampling must be performed in months when the endophyte fully expresses itself in the fescue plant, which generally occurs when plants have been growing well for at least a month. From an experiment conducted to compare monthly infection rates of tall fescue pastures in Georgia, it has been reported that infection rates were stable when sampled from late April through January.

Identification of a Tall Fescue Pasture

The pictures on the right side and the following characteristics could be used to identify a tall fescue pasture:

Growth Habitat:

- Cool season, perennial bunch grass. Grass has a tendency to form a very tight sod.

Plant Characteristics:

- Bunch growth habitat but forms sod by rhizomes when mowed or grazed frequently.
- Leaf blades are flat and have rough margins and a prominent midrib.
- Blades are dull and ribbed on the upper surface and glossy below.
- Younger leaves are rolled in the whorl.
- Flowering stems are round and smooth and grow mostly erect.
- The grass usually grows between 24 and 48 inches tall.

The Supplied Sampling Kit:

8. An Insulated Foam Shipping Kit - 6" x 4.5" x 3"
9. A Cold Pack (Ice Brick): 5.5" x 4" x 0.75"
10. A quart size Zip-lock bag with submission form in it
11. A gallon size Zip-lock bag with a piece of paper towel in it, to be used for sample
12. A Prepaid UPS shipping label to Send the Sample to The Feed and Environmental Laboratory

Place the Cold Pack (Ice Brick) in a freezer at least a day before taking the sample to ensure it is frozen during sending the sample.

Selecting a Stand to be Sampled

Select a fescue stand the same seeding date and management for collecting one sample which will contain 30 tillers. To ensure representativeness of the sample, avoid collecting from:

1. Ditches, pond areas, winter feeding sites and borders, unless these areas make up more than 20 percent of the stand.
2. Dry, rocky areas, or areas with low fertility because endophyte infection provides the fescue with increased hardiness.
3. Additionally, manure and urine spots will not provide an accurate result and should not be sampled. These locations typically have higher soil nitrogen concentrations, which may lead to a biased result.

Note: New tall fescue varieties such as MaxQ and others contain a novel or non-toxic endophyte. that cannot be distinguished from other infected stands using currently available commercial laboratory procedures. As the goal of this project is to evaluate the nature and extent of toxic endophyte infection, there is no need to sample fields that are known to be novel-endophyte varieties.

Number of Tillers to be Collected: 30.

A Helpful Video for Sampling is Available Here: https://youtu.be/s6eF4XBbv7g

Sampling Spots Within the Stand for a Representative Sample

It is critical that the specimens collected be representative of the field at large. Make sure you take your 30 tiller samples in an appropriate way so you get roughly the same number of tillers from all sections of the field. The specimens should be taken at random, by walking a zigzag or circular pattern around the field (See **Figure 1**).

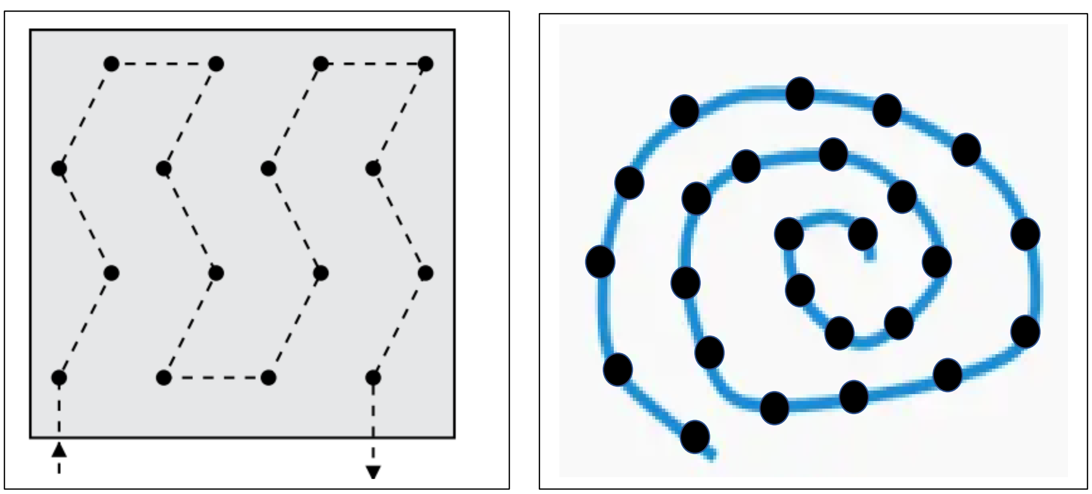

Figure 1. Collect tillers randomly from various sampling spots using a zig-zag (left) or circular (right) pattern.

Collecting the Tillers

1. Prepare the enclosed zip-top plastic bag by placing a damp (not wet!) paper towel in it to help keep freshness (i.e., to prevent drying) of the tiller samples to be collected.
2. To sample, identify a fescue plant (i.e., a crown/clump/bunch with multiple tillers) around the sampling spot and select one healthy tiller (stem) from that plant (crown). **Only one tiller should be collected from each selected fescue plant (i.e., a crown/clump/bunch).** Tillers — greater than 1/8 inch in diameter — provide the best sampling material. Here are few important points to consider:
    - Sample only vegetative tillers, avoiding plants in the boot stage with emerging heads or with mature seed heads.
    - Select tillers that are of average size for the field.
    - Avoid selecting extra-large or extra-small tillers.
    - Do not select small, emerging, or unhealthy tillers.
    - Avoid plants with soil or fecal contamination.
    - Ensure that any crown/clump/bunch is sampled only once.
3. Follow the selected tiller to its union with the crown and cut off the tiller at the ground surface. The tiller samples should be cut low enough that some remnant roots are present.
4. Trim away any excess leaf from the sample and clip the tiller keeping a total length of about 4 inches from the base, being sure the base of the tiller remains intact (See **Figure 2**).

Figure 2. Sampling fescue tillers (left). Healthy stems are cut at the base of the plant at the soil surface. Leaf tissue above the collar (right) can be removed. Then the tiller should immediately put in a cool, damp environment.

5. Place tiller in the zip-top plastic with the damp paper towel in it.
6. Complete collection of 30 tillers, one from each of all 30 sampling spots, in the sampling unit.
7. Fill out the enclosed sample submission form provided in another zip-top bag. Place the filled submission form back to the zip-top bag.
8. Place the two zip-top bags one with samples and another with submission form in the styro-foam box and place Cold Pack (Ice Brick) in the styro-foam box to maintain a cool environment. Ship it overnight using the enclosed prepaid shipping label to:

Feed and Environmental Water Laboratory, University of Georgia, 2300 College Station Road Athens, GA 30602

(Do not Ship the Samples Thursday and Friday of the Week)