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Sampling Tall Fescue for Endophyte Infection and Ergot Alkaloid Concentration

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Introduction

Tall fescue (*Festuca arundinacea* syn *Lolium arundinaceum syn Schedonorus phoenix*) is a productive cool-season grass and the predominant forage in Virginia. The majority of tall fescue grown in Virginia contains a fungal endophyte (*Epichloë coenophiala* ex. *Neotyphodium coenophialum*) — a fungus that helps the host plant cope with abiotic (e.g., drought and flood) and biotic (e.g., insect and grazing animal) stressors. However, toxic compounds (ergot alkaloids) produced by this endophyte can reduce grazing animal performance.

Fescue toxicosis is the general term describing three disorders related to the consumption of ergot alkaloids: fescue foot, fat necrosis, and summer syndrome or summer slump. Specific production losses from fescue toxicosis result from poor reproductive performance, reduced weight gain, and lower milk production. For further information regarding the effects of and management strategies for tall fescue toxicosis, see VCE publication 418-050, "Making the Most of Tall Fescue in Virginia."

Understanding both the risks posed and the management needed to address fescue toxicosis issues on the farm is often best facilitated by assessing fungal infection levels in pastures. Two different tests are required to determine both the presence of the endophyte and the level of alkaloids in tall fescue. Information gained by testing pastures can help producers develop grazing and mitigation strategies to avoid severe incidences of fescue toxicosis. Knowing endophyte levels and typical alkaloid concentrations across the farm can help producers make decisions about grazing management (e.g., which animals go where) and feeding and supplementation strategies that could reduce the potential production losses associated with the toxin burden. Repeated testing during a grazing season can help determine the possible benefits of pasture renovation or the addition of legumes.

The purpose of this publication is to provide information on how to sample and test fescue for endophyte infection and ergot alkaloid content. Infection levels can tell how much of the fescue in a pasture carries the endophyte, but they will not tell the concentration of alkaloids in the fescue. In most cases, however, high levels of endophyte presence will be associated with high levels of alkaloid intake.

When to Sample and Methods for Determining Endophyte Presence

Endophytes are dormant in the winter and break dormancy a few weeks after fescue begins active growth. Early spring sampling could reduce the accuracy of test results because the endophyte does not become active in the plant until after spring green-up. For this reason, it might be better to sample for endophyte infection once the fescue seedhead has emerged on plants that are healthy and green. In most parts of Virginia, seedheads develop in the latter part of May and remain through mid-July if undisturbed. Testing for endophyte presence can occur from this point into the fall, although presence of the seedhead (in spring) can aid fescue identification (fig. 1).

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Figure 1. Tall fescue can be seen in bunches but has short rhizomes, allowing it to spread and form sod (top left). The seedhead is a tight panicle early in development that then begins to open (top right). Leaves are ribbed and do not wrap around the stem (bottom left). Leaf tips are marked by a constriction (circle; bottom right). (Photos by Marie Rothwell and John Fike.)

Plant samples are analyzed for endophyte presence either through staining or immunoblot techniques that indicate the presence of fungal proteins. With staining methods, fresh plant tissues are cut and treated with a stain that attaches to the endophyte. The fungus can then be detected when viewed under a microscope (fig. 2).

The immunoblot technique is essentially a staining technique that doesn't require microscopic observation. Presence of the endophyte is detected indirectly when antigens to the fungus are bound by a stain (which is similar to how a home pregnancy test works). Results from either method of analysis are accurate for determining endophyte infection. Regardless of the laboratory analysis used, proper sampling techniques should be followed. Therefore, it is important to obtain representative samples that will accurately describe endophyte presence for the fields in question.

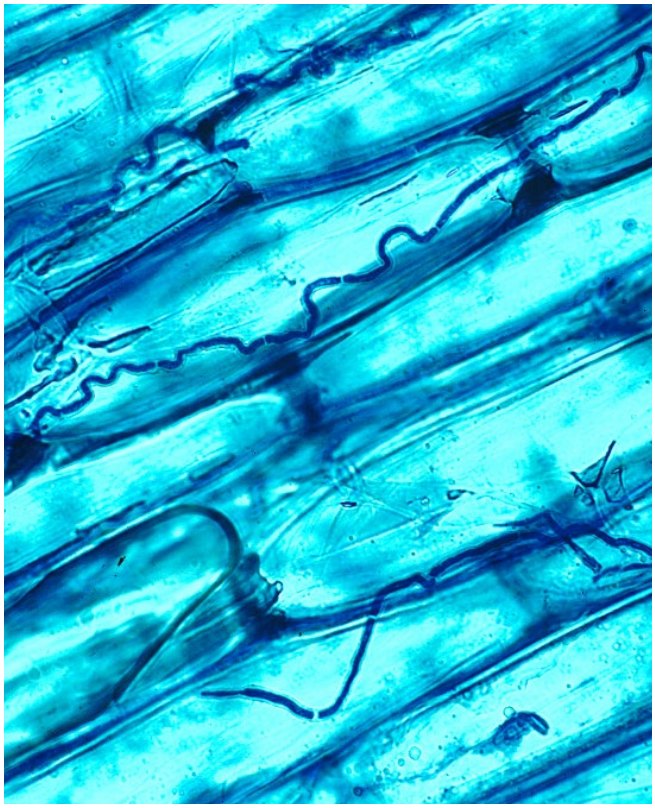

Figure 2. Fungal mycelia in tall fescue (blue stained lines indicated by the arrows) live between the cells of the fescue plant. (Photo by Nick Hill, courtesy of USDA-ARS.)

How to Sample for Endophyte Presence in Fescue Pastures

Only fescue plants must be selected for endophyte analysis. This requires well-developed grass identification skills because distinguishing grasses can be difficult, especially when they are in a vegetative state. Annual and perennial ryegrasses, quackgrass, and other species can be mistaken for fescue. Furthermore, sampling should be done on living plant tissue to provide reliable data. Dried or decaying leaves and stems will not have active endophytes.

Tillers (the stem plus leaves of fescue plants are collected for endophyte analysis. Plants (and thus, tillers) sampled for endophyte presence should be representative of the entire pasture. Collect tiller samples from 20 to 50 different plants in random locations throughout the entire field and avoid areas that are not representative of the pasture as

a whole. Fescue is often the predominant species on difficult sites, such as dry, rocky areas, winter feeding areas, or areas with low fertility because endophyte infection provides the fescue with increased hardiness. Oversampling in these areas might not be representative of the pasture as a whole.

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 (fig. 3).

Figure 3. Pastures often have nutrient "hot spots". Where possible, avoid sampling areas that are near urine patches and manure pats, or that appear to have clumped growth (from previous urine or fecal deposits). This can lead to an unrepresentative sample. (Photo by John Benner.)

To sample (fig. 4), identify a fescue plant and select one healthy tiller (stem) from that plant. Do not collect small, emerging, or unhealthy tillers. Follow the tiller to its union with the crown and cut off the tiller at the ground surface. Trim away any excess leaf from the sample and clip the tiller to a total length of about 4 inches, being sure the base of the tiller remains intact. Immediately transfer all tillers into a zip-top plastic bag and keep cool throughout sampling and shipping. Enclose a damp (not wet!) paper towel in the plastic bag to help keep samples fresh. Large tillers — greater than 1/8 inch in diameter — provide the best sampling material.

Figure 4. 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 plant should immediately put in a cool, damp environment. (Photos by John Benner and John Fike.)

Ergot Alkaloid Levels in Tall Fescue

Alkaloid concentrations are a function of plant growth. Because the plant supplies nutrients to the fungus, alkaloid levels typically are highest in spring and fall (generally May or June and September or October in Virginia) when plants are growing fastest. Along with environmental conditions (e.g., temperature and rainfall), ergot alkaloid concentrations in fescue plants are affected by factors such as soil fertility and plant and endophyte genetics. Pasture alkaloid levels can

vary widely among fields and both throughout and between growing seasons because of the interactions among these factors. In highly infected pastures, total ergot alkaloid concentrations often range from a few hundred to 1,000 parts per billion (ppb) or more.

A study tracking the fescue alkaloid concentrations of nine pastures in the Shenandoah Valley of Virginia during the 2014 growing season revealed levels ranging from 531 to 7,500 ppb. Measurable negative effects of alkaloids on grazing livestock have been reported with alkaloid concentrations as low as 500 ppb (Craig et al. 2015). As fescue plants mature, the endophyte grows up into the reproductive stem, and alkaloid concentrations in the seedhead rise dramatically. This process allows the fungus to propagate and spread with the seeds.

Local sampling in the Shenandoah Valley has indicated that plants tend to have the highest alkaloid concentrations at early heading. Concentrations often decline during summer when fescue growth slows dramatically. Despite this, however, the most observable negative impact of tall fescue toxicosis in Virginia occurs during the summer months, when hotter temperatures compound the vasoconstrictive effects of the alkaloids. That is, the alkaloids constrict blood vessels, decreasing the animal's ability to dissipate heat at the time when it is most heat-stressed. Alkaloid production and corresponding tissue concentrations increase when fescue resumes rapid growth with cooler temperatures in late summer and early fall (Rogers et al. 2011).

Sampling for Ergot Alkaloids

Determining the presence or absence of endophyte is helpful for managing fescue pastures across a farm, but by itself, it does not reveal the level of toxins available to grazing livestock. Measuring actual ergot alkaloid concentrations in fescue provides a more comprehensive picture of pasture toxicity than simply knowing what percent of the fescue plants in a pasture are infected.

Plant tissue collection required to determine alkaloid concentrations is similar to testing for the endophyte. Testing can be expensive, so sampling should be planned to answer objective questions regarding fescue toxicity. Generally, it is preferable to sample only fescue even when pastures are diverse and contain many species. Consider objectives and farm management when prioritizing fields to sample or dates when samples will be taken. Keep in mind the following when planning to sample your farm for endophyte and alkaloid levels:

•Prioritize sampling decisions on when the pastures are usually grazed and what types of animals will graze them. For example, pastures to be grazed by breeding animals in June would have higher priority than pastures primarily used for winter grazing.

•If sampling over time (by season or year), estimate and record the levels of fescue in pastures to serve as a useful reference point for observed toxicosis issues.

•If whole sward sampling is preferred, consider using a quadrat or frame to clip and objectively collect all forage within a given location (fig. 5).

•If the objective is to estimate alkaloid levels in the diet of grazing animals, an effort should be made to mimic the animals' selection of plant parts. "Hand-plucked" or "grab" samples can be collected by grasping the forage and twisting and plucking it similar to the manner of a grazing animal. Before sampling, it may help to observe animals grazing the field to be sampled. Pastures under rotational management can be sampled either prior to animals' entry or while animals are grazing.

•A consistent sampling method is essential if the objective is to compare alkaloid levels among pastures. Time of year, location in the field, plant parts, growth stage, and time of sampling relative to grazing events are important to consider for alkaloid testing and comparison.

•High nitrogen fertility can greatly increase alkaloid production; avoid sampling fescue growing near manure or urine patches.

•The sample that will be sent for testing should be a composite of forage tissue collected from 20 to 30 locations throughout the field. Walking from fencerow to fencerow in a zigzag or "W" pattern can be helpful for getting representative samples from across a pasture.

• Alkaloids degrade significantly after harvest as the sampled forage dries. Keep samples as fresh as possible by bagging and placing in a well-iced cooler immediately after collection.

Figure 5. A quadrat frame for collecting whole sward samples. (Photo by Matt Booher.)

Where to Send Samples

Samples can be tested for a fee at several different laboratories throughout the Southeast. Some labs have the capacity to determine both endophyte presence and total alkaloid concentrations. Contact the laboratory to obtain its sample handling recommendations as well as the best times to mail a sample.

Information on sample information, handling, and fees can be obtained from the following laboratories:

Agrinostics Ltd. Co.
info@agrinostics.com
www.agrinostics.com/index.html

Auburn University
Fescue Diagnostic Laboratory
www.ag.auburn.edu/enpl/services/fescue.htm

North Carolina Dept. of Agriculture and Consumer Services
Plant Industry Division, Seed Section
Endophyte Testing Service
919-707-3736
www.ncagr.gov/plantindustry/seedandfertilizer/seed/Endophyte.htm.

University of Kentucky
Seed Laboratory, Division of Regulatory Services
859-218-2468
www.rs.uky.edu/seed/ServiceTesting/howto_submitsamples.php

University of Kentucky
Veterinary Diagnostic Laboratory
www.vdl.uky.edu/Home.aspx
www.vdl.uky.edu/TestInformation.aspx
(Click "Toxicology," select "Ergovaline")

Using the Results

Sampling tall fescue for endophyte infection and alkaloid concentrations will reveal levels of toxicity in high-percentage fescue pastures. Once the toxicity of a pasture is known, a plan can be developed to manage and reduce alkaloid intake by grazing animals through rotation, supplementation, and renovation strategies. Acute fescue toxicity/summer slump is often observed in high-percentage fescue fields in which competing forages make up a small percentage of intake. If alkaloid levels are high enough, the field could be a candidate for replacement with nontoxic novel endophyte. If alkaloid levels are more manageable, overseeding clover and other forages to dilute the fescue toxins and diversify animal intake might be an option. Management strategies are considered in VCE publication 418-050, "Making the Most of Tall Fescue in Virginia."

Summary

Endophyte-infected tall fescue is the dominant forage in Virginia. As such, it is imperative to manage this important forage resource wisely. Ergot alkaloids produced by fescue's fungal endophyte create challenges to accomplishing this. Tests for endophyte presence and alkaloid levels are important management tools that producers can use to minimize alkaloid consumption and the negative impacts on animal performance. Consistent testing methods are important for adequately assessing alkaloid levels and for making comparisons among pastures over time. These results can then be used to develop a custom grazing strategy to avoid severe incidences of fescue toxicosis. Repeated testing during a grazing season can help determine possible benefits to pasture renovation or the addition of legumes. Similar to testing forages for nutrient concentrations and devising a winter feeding and supplement plan, testing fescue-based pastures for endophyte infection level and for ergot alkaloid concentrations at various times during the year can facilitate management to reduce alkaloid consumption and also help determine if further mitigation is needed.

References

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Rogers, Wendi M., Craig A. Roberts, John G. Andrae, David K. Davis, George E. Rottinghaus, Nicholas S. Hill, Robert L. Kallenbach, and Don E. Spiers. 2011. "Seasonal Fluctuation of Ergovaline and Total Ergot Alkaloid Concentrations in Tall Fescue Regrowth." *Crop Science* 51 (3): 1291-96.