Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) is one of the most widely grown grasses in the United States. Most plants are infected with an endophyte, *Epichloë coenophiala* Bacon and Schardl. This endophyte produces high concentrations of ergot alkaloids, which cause fescue toxicosis in livestock. The economic losses to the US livestock industry are estimated to be approximately $1 billion annually. The objectives were to evaluate the variability in total ergot alkaloid concentrations ([TEA]) and ergovaline concentrations ([E]) in leaf blades and sheaths, and whole tillers from cultivars containing different wild-type and novel endophytes across two seasons and environments. This study was conducted in 2012 and 2014 at two sites: Watkinsville, GA and Lexington, KY. There was no location effect for [TEA] or [E] in whole tillers. Measurements in 2012 showed no ergovaline-producing tillers for cultivars infected with MaxQ or MaxQ II. Cultivars BarOptima Plus E34 and IS-FTF 31-UArk9 produced tillers expressing ergovaline but at lesser levels than endophyte-infected KY 31. In leaf blades, [TEA] and [E] were greater in 2012 than 2014 at both sites for KY 31 (1861 and 402 vs. 1473 and 135 µg kg⁻¹, respectively). Large variability can occur in KY 31, BarOptima Plus E34, and IS-FTF 31-UArk9 for ergot alkaloid content over years, within seasons, and between cultivars. This research indicates the importance of testing fields for ergot alkaloid content to determine risk from grazing and hay feeding.
Though there are several ergot alkaloids produced by tall fescue endophyte-plant associations, ergovaline has been found to be present in the highest concentration, constituting 10% to 50% of the total ergot alkaloid concentration ([TEA]) and 85% to 97% of the ergopeptide alkaloids (Lyons et al., 1986). As a result of its high abundance, ergovaline was implicated early on as being the primary cause of fescue toxicosis (Belesky et al., 1988; Klotz and Nicol, 2016). Ergovaline concentration ([E]) thresholds have been proposed as an indicator of fescue toxicity risk. Using case studies and range-finding experiments, Tor-Agbidye et al. (2001) proposed dietary levels of ergovaline equal to or greater than 400 to 750 µg kg⁻¹ and 500 to 800 µg kg⁻¹ can be expected to result in fescue toxicosis in cattle (Bos taurus) and sheep (Ovis aries), respectively. Similarly, Craig et al. (2014) examined additional case studies to refine those ranges to set [E] threshold values used by the Oregon State University Endophyte Service Laboratory of 300 to 500 µg kg⁻¹ for non-pregnant horses and cattle and 500 to 800 µg kg⁻¹ for sheep. Liebe and White (2018) conducted a meta-analysis of beef cattle performance research and found that animal performance was estimated to reach a break point at 60 µg kg⁻¹, since average daily gain (ADG) when [E] in the diet remained less than 60 µg kg⁻¹ was 40% greater than when [E] was greater than 60 µg kg⁻¹. However, the dataset used in the meta-analysis had relatively few data points from situations where [E] was between 50 µg kg⁻¹ and 800 µg kg⁻¹, so actual thresholds are likely closer to those proposed by Tor-Agbidye et al. (2001) or Craig et al. (2014).

With the introduction of non-ergot alkaloid-producing endophytes into tall fescue cultivars (Bouton et al., 2002), tall fescue varieties are available that are non-toxic, sustain livestock performance equal to endophyte-free analogs, and produce the secondary metabolites that protect the plant against abiotic and biotic stress and aid stand persistence (Parish et al., 2003, Nihsen et al., 2004). These new varieties are marketed as “non-toxic,” “novel,” or as non-ergot alkaloid-producing endophyte-infected tall fescue cultivars across two distinct seasons and environments. The hypothesis was that wild-type endophytes would have [TEA] and [E] greater than novel endophytes, but specific concentrations would vary throughout the growing season.

**MATERIALS AND METHODS**

These studies were conducted in 2012 and 2014 in conventionally-tilled, newly established tall fescue plots at two sites: the University of Georgia Plant Sciences Farm located west of Watkinsville GA (33°52′ N and 83°31′ W, and elevation 257 m) on a flat Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapludults); and the University of Kentucky Spindletop Farm at Lexington, KY (38°07′ N and 84°29′ W, and elevation 281 m) on a gently sloping Bluegrass-Maury silt loam (Fine, mixed, active, mesic Typic Paleudalfs). The two sites represent a northerly and southerly latitude within the transition zone (tall fescue belt; Roberts and Andrae, 2004). The Georgia site had previously been planted to wheat (Triticum aestivum L.), and the Kentucky site was previously orchardgrass (Dactylis glomerata L.).

The tall fescue treatments included a set of three cultivars that expressed ergot alkaloids including endophyte-infected Kentucky 31 (KY31-EI, University of Kentucky, Lexington, KY); BarOptima Plus E34 (Barenbrug USA, Tangent, OR); and IS-FTF 31-UArk9 (DLF International Seeds, Halsey, OR); and a second set of three cultivars that contain non-ergot alkaloid-producing endophyte E. coenophiala, including Jesup MaxQ (Pennington Seed, Lebanon, OR); Lancefield MaxQ II (University of Kentucky, Lexington, KY); and Texoma MaxQ II (Noble Research Institute, Ardmore OK). Seeds for this trial were supplied directly by the institution that bred or the company that owns and/or licenses the cultivar and stored in a cooler (5°C) until planting. A tall fescue treatment containing nil endophyte (KY31-EF) was created by heat-treating seeds of KY31-EI at a temperature of 47°C in a high-humidity chamber (achieved by addition of 75:25 glycercol/water [v/v] to chamber) for 14 d at the Noble Research Institute (Bouton et al., 1993). Before the plots of tall fescue were planted in autumn 2011, 50 seeds of each entry were analyzed by the Forage Research Laboratory in the Department of Crop and Soil Sciences, University of Georgia, Athens, GA for germinability and 100 seed lots for endophyte infection percentage (EI%), [TEA], and [E] could verify they are non-toxic in a more cost-effective protocol than by conducting grazing trials.

The [TEA] and [E] in toxic tall fescue is the lowest in leaf blades, greater in leaf sheaths, and highest in seed heads (Lyons et al., 1986; Rottinghaus et al., 1991), and these levels are affected by season, weather, grazing management, leaf age, fertilization, and other growing conditions (Lyons et al., 1986; Belesky et al., 1988; Rottinghaus et al., 1991; Agee and Hill, 1994; Belesky and Hill, 1997; Malinowski et al., 1998; Rogers et al., 2011; Kenyon et al., 2018). It is not known how these environmental factors may affect the endophyte-cultivar combinations marketed as “non-toxic,” “novel,” or as “non-ergot alkaloid-producing.” It is also unknown whether the [TEA] and [E] of these cultivars are stable within and across seasons. Therefore, the objectives of this study were to evaluate the variability in [TEA] and [E] in leaf blades, leaf sheaths, and whole tillers from wild-type, endophyte-free, non-toxic, novel, and non-ergot alkaloid-producing endophyte-infected tall fescue cultivars across two distinct seasons and environments. The tall fescue treatments included a set of three cultivars that contained non-ergot alkaloid-producing endophyte E. coenophiala, including Jesup MaxQ (Pennington Seed, Lebanon, OR); Lancefield MaxQ II (University of Kentucky, Lexington, KY); and Texoma MaxQ II (Noble Research Institute, Ardmore OK). Seeds for this trial were supplied directly by the institution that bred or the company that owns and/or licenses the cultivar and stored in a cooler (5°C) until planting. A tall fescue treatment containing nil endophyte (KY31-EF) was created by heat-treating seeds of KY31-EI at a temperature of 47°C in a high-humidity chamber (achieved by addition of 75:25 glycercol/water [v/v] to chamber) for 14 d at the Noble Research Institute (Bouton et al., 1993). Before the plots of tall fescue were planted in autumn 2011, 50 seeds of each entry were analyzed by the Forage Research Laboratory in the Department of Crop and Soil Sciences, University of Georgia, Athens, GA for germinability and 100 seed lots for endophyte infection percentage (EI%), [TEA], and [E] could verify they are non-toxic in a more cost-effective protocol than by conducting grazing trials.

**Trial Design and Management**

The tall fescue treatments were drilled using a Sukup no-till drill (Sukup, Sheffield, IA) fitted with a Wintersteiger cone seeder (Wintersteiger AG, Reid im Innkreis, Austria) into conventionally-prepared seed beds at a rate of 22.4 kg of pure live seed ha⁻¹.
Sample Collections
In April 2012, June 2012, and June 2014, 10 to 20 tall fescue tillers per plot were sampled and analyzed for infection with *E. coenophiala* by immunoassay and for ergot-alkaloid producing endophyte by immunosorbent assay (ELISA; Agrinostics Ltd. Co., Watkinsville, GA; Hiatt and Hill, 1997) to determine infection rates of tillers. Tall fescue tiller samples were also taken to determine [TEA] and [E] at each site. At the Georgia site, samples were collected on eight occasions from April through November 2012 and repeated on eight occasions from March through November 2014. At the Kentucky site, tiller samples were collected on eight occasions from April through November 2012, and repeated on eight occasions from April through December 2014. The timing of collections was determined when regrowth of tall fescue plants had attained three to four leaves and was different between sites due to different climatic conditions. Samples of tillers were cut to a residual height of 25 mm at twenty random positions within each plot and combined. These samples were sealed in plastic bags and placed in an insulated container with ice for 3 h prior to freezing at –25°C. A second set of samples was taken from the 20 positions in each plot for tiller dissection, stored temporarily in an insulated container, dissected and separated to create fractions containing sheaths and blades, and stored at –25°C. Fresh plant materials were frozen (–25°C) and then lyophilized and ground through a 1-mm screen using a Cyclotec 1093 Sample Mill (Foss, Eden Prairie, MN). The ground samples were analyzed for total ergot alkaloid concentration by ELISA by Agrinostics Ltd. Co. (Watkinsville, GA) using the methods of Agee and Hill (1994). They were also analyzed for [E] by high performance liquid chromatography (HPLC) by Dr. George Rottinghaus, Veterinary Medicine Diagnostic Laboratory at the University of Missouri, following the procedure of Rottinghaus et al. (1991) with modifications reported by Hill et al. (1993). Total monthly precipitation data in Lexington, KY and Watkinsville, GA during the establishment year (2011) through the final growing season (2014) are provided in Table 3. Average ambient temperature data in Lexington, KY and Watkinsville, GA during the two harvest years (2012 and 2014) are provided in Table 4.

**Statistical Analysis**
The data for percentage seed infection were modelled using a Bernoulli generalized linear model with a logit link in a Bayesian framework (Chen and Shao, 1999). Model parameters were given normal (0, sd = 5) priors. Tiller infection rates and [TEA] and [E] were analyzed using analyses of variance with the PROC GLM procedure in SAS, with site, year, and their interaction as fixed effects. When analysis of variance showed significant differences, mean comparisons were made using least significant difference at the 0.05 level of significance.
Table 4. Average ambient temperature in Lexington, KY and Watkinsville, GA in 2012 and 2014†.

<table>
<thead>
<tr>
<th></th>
<th>Georgia 2012</th>
<th>Georgia 2014</th>
<th>Kentucky 2012</th>
<th>Kentucky 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>°C</td>
<td>°C</td>
<td>°C</td>
</tr>
<tr>
<td>January</td>
<td>8.3</td>
<td>9.4</td>
<td>3.3</td>
<td>–3.9</td>
</tr>
<tr>
<td>February</td>
<td>9.4</td>
<td>7.8</td>
<td>4.4</td>
<td>–1.1</td>
</tr>
<tr>
<td>March</td>
<td>17.2</td>
<td>10.6</td>
<td>13.3</td>
<td>3.9</td>
</tr>
<tr>
<td>April</td>
<td>17.8</td>
<td>16.1</td>
<td>13.3</td>
<td>15.4</td>
</tr>
<tr>
<td>May</td>
<td>22.2</td>
<td>20.6</td>
<td>20.6</td>
<td>18.9</td>
</tr>
<tr>
<td>June</td>
<td>23.9</td>
<td>24.4</td>
<td>22.8</td>
<td>23.9</td>
</tr>
<tr>
<td>July</td>
<td>26.7</td>
<td>26.3</td>
<td>27.2</td>
<td>23.3</td>
</tr>
<tr>
<td>August</td>
<td>23.9</td>
<td>25.8</td>
<td>23.9</td>
<td>24.4</td>
</tr>
<tr>
<td>September</td>
<td>27.2</td>
<td>27.3</td>
<td>24.3</td>
<td>24.4</td>
</tr>
<tr>
<td>October</td>
<td>16.1</td>
<td>17.2</td>
<td>12.8</td>
<td>13.9</td>
</tr>
<tr>
<td>November</td>
<td>10.6</td>
<td>8.3</td>
<td>6.1</td>
<td>5</td>
</tr>
<tr>
<td>December</td>
<td>9.4</td>
<td>8.9</td>
<td>5.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

§ Dashes indicate infection rate was not determined.

RESULTS AND DISCUSSION

Infection of Seeds and Tillers with *Epichloë coenophiala*

Since the same seed lots were used for both locations, data are pooled across locations. Seeds of Texoma MaxQ II, and Lacefield MaxQ II were highly infected with *E. coenophiala* (Table 5). Seeds of Jesup MaxQ and BarOptima Plus E34 were also highly infected with *E. coenophiala*, but tillers had lesser live, viable infection rates than KY31-EI and the MaxQ II infected cultivars. IS-FTF 31-UArk9 contained the least amount of seeds infected with live, viable *E. coenophiala*. Ergot alkaloid-producing endophytes were found at low infection levels in seeds of IS-FTF 31-UArk9 and BarOptima Plus E34 (10% and 20% respectively), while none were found in the MaxQ and MaxQ II infected cultivars. Tillers of KY31-EI were highly infected with live, viable *E. coenophiala* endophyte.

The response of field tiller infection rates differed (*P* = 0.02) with location and year, but no interaction was observed, so tiller infection rates are presented separately by year and location. In 2012, KY31-EI, Lacefield MaxQ II, and Texoma MaxQ II had greater (*P* ≤ 0.02) percentages of infected tillers than IS-FTF 31-UArk9, Jesup MaxQ, and KY31-EF in Georgia (Table 6). BarOptima Plus E34 contained intermediate values, and was not different from Lacefield MaxQ II, Texoma MaxQ II, or Jesup MaxQ (0.07 ≤ *P* ≤ 0.58). During the 2012 growing season in Kentucky, Lacefield MaxQ II, Texoma MaxQ II, and KY31-EI had greater (*P* ≤ 0.01) percentages of infected tillers than all other varieties except BarOptima Plus E34. Jesup MaxQ contained fewer (*P* ≤ 0.03) infected tillers than Lacefield MaxQ II, Texoma MaxQ II, and KY31-EI. IS-FTF 31-UArk9 had approximately 70% fewer (*P* < 0.0001) infected tillers than the varieties with the highest rates, while KY31-EF had only 1% infected tillers. Jesup MaxQ, Lacefield MaxQ II, and Texoma MaxQ II were not sampled for infected tillers in 2014, since none (0%) of the tillers were producing ergot alkaloids at the initial sampling at both sites (Table 7). Of the tested varieties in 2014 at the Georgia site, KY31-EI had the greatest (*P* < 0.0001) amount of infected tillers. IS-FTF 31-UArk9 and BarOptima Plus E34 had median values, with IS-FTF 31-UArk9 being 16% greater (*P* = 0.04) than BarOptima Plus E34. Similar to 2012, no infected tillers were observed in KY31-EF. Parish et al. (2003) observed tiller infection rates of at least

Table 5. Percentage of seeds and tillers of six varieties of tall fescue tested for *Epichloë coenophiala* and expression of ergot alkaloids in 2011 prior to drilling the trials. Values are the observed percentages with 95% highest posterior density credible intervals (Chen and Shao, 1999) in parentheses.

<table>
<thead>
<tr>
<th>Tall fescue variety and strain of endophyte</th>
<th>Lot Number</th>
<th>% Seed infected with live + dead <em>E. coenophiala</em></th>
<th>% Seed infected with ergot alkaloids</th>
<th>% Tillers† with live, viable <em>E. coenophiala</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>BarOptima Plus E34</td>
<td>BWC–11–06–03</td>
<td>80b ‡ (72–88)</td>
<td>20b (11–27)</td>
<td>81c (73–89)</td>
</tr>
<tr>
<td>IS-FTF 31-UArk9</td>
<td>B44–11–RS–10</td>
<td>56c (46–67)</td>
<td>10c (4–16)</td>
<td>34d (26–44)</td>
</tr>
<tr>
<td>Jesup MaxQ</td>
<td>Y28–10–MQ9</td>
<td>96a (92–99)</td>
<td>0d (0–1)</td>
<td>86c (79–92)</td>
</tr>
<tr>
<td>Lacefield MaxQ II</td>
<td>C2.09.NxGn</td>
<td>97a (94–100)</td>
<td>0d (0–1)</td>
<td>96b (92–99)</td>
</tr>
<tr>
<td>Texoma MaxQ II</td>
<td>NFTF–144–07</td>
<td>98a (95–100)</td>
<td>0d (0–1)</td>
<td>96b (92–99)</td>
</tr>
<tr>
<td>KY31-EI</td>
<td>10.34.Bik3.3</td>
<td>–§</td>
<td>97a (93–100)</td>
<td>100a (99–100)</td>
</tr>
<tr>
<td>KY31-EF</td>
<td>10.34.Bik3.3</td>
<td>–§</td>
<td>–§</td>
<td>3a (1–6)</td>
</tr>
</tbody>
</table>

† Plants were grown out from seed of the six varieties/endophyte strains in a greenhouse and 100 plants of each combination were obtained for immunoblot analyses.
‡ Within a column, means without a common letter differ significantly. Pairwise differences in percentage infected were deemed significant if the probability that the percent infected for plant type A was larger than the percentage infected for plant type B was over 0.96.
§ Dashes indicate infection rate was not determined.
68 and 80% in non-ergot alkaloid-producing tall fescue and KY31-EI, respectively, at two locations in Georgia, similar to the current study. At the Kentucky site in 2014, KY31-EI again had the greatest \( P \leq 0.01 \) percent of infected tillers and KY31-EF the least \( P \leq 0.01 \). BarOptima Plus E34 had lesser \( P \leq 0.01 \) infected tiller rates than KY31-EI, but was greater \( P < 0.0001 \) than IS-FTF 31-UArk9. Inconsistent results have been reported on the effects of increased temperature on tiller infection rates. McCulley et al. (2014) reported increasing temperature by 3°C (similar to the ambient temperature difference observed between the Georgia and Kentucky locations; Table 4) had no effect on infected tiller percentage. However, in the current study averaged across varieties, Kentucky ranked lesser than Georgia in rate of infected tillers. Furthermore, at both locations, IS-FTF 31-UArk9 was the only variety to have a numerical increase in infected tillers from 2012 to 2014. This is likely due to loss of endophyte–free plants in those plots.

The effect of the tall fescue variety on the percentage of field tillers expressing [TEA] differed \( P < 0.0001 \) by year. In 2012, KY31-EI contained a greater \( P < 0.0001 \) proportion of tillers expressing total ergot alkaloids than any other variety (Table 7). BarOptima Plus E34 and IS-FTF 31-UArk9 had 15% and 80%, respectively, fewer tillers \( P < 0.0001 \) expressing total ergot alkaloids than KY31-EI. Jesup MaxQ, Lacefield MaxQ II, and Texoma MaxQ II had no tillers \( P < 0.0001 \) expressing ergot alkaloids, despite each having high concentrations of \( E. coenophiala \) infected tillers. Since KY31-EF did not have \( E. coenophiala \) infected tillers, it also had no tillers expressing total ergot alkaloids. The ranking of these varieties in terms of ergot alkaloid-producing tiller percentages was similar in 2014, but IS-FTF 31-UArk9 seemed to have a greater proportion of such tillers in 2014, with 29% endophyte infection producing 211% more total ergot alkaloids. As with the tiller infection rates, we hypothesize that this increase was the result of the loss of endophyte–free tillers between 2012 and 2014.

### Table 6. Seasonal mean percentage of field tillers of six varieties of tall fescue sampled in 2012 and 2014 at the Georgia and Kentucky sites expressing \( E. coenophiala \).

<table>
<thead>
<tr>
<th>Tall fescue variety/Strain of Endophyte</th>
<th>Georgia</th>
<th>Kentucky</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
<td>2014†</td>
</tr>
<tr>
<td>BarOptima Plus E34</td>
<td>86b,‡</td>
<td>70c</td>
</tr>
<tr>
<td>IS-FTF 31-UArk9</td>
<td>43d</td>
<td>81b</td>
</tr>
<tr>
<td>Jesup MaxQ</td>
<td>74c</td>
<td>—</td>
</tr>
<tr>
<td>Lacefield MaxQ II</td>
<td>90a,b</td>
<td>—</td>
</tr>
<tr>
<td>Texoma MaxQ II</td>
<td>93a,b</td>
<td>—</td>
</tr>
<tr>
<td>KY31-EI</td>
<td>100a</td>
<td>96a</td>
</tr>
<tr>
<td>KY31-EF</td>
<td>0e</td>
<td>0d</td>
</tr>
<tr>
<td>SEM</td>
<td>5.9</td>
<td>3.9</td>
</tr>
<tr>
<td>( P )-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

† Presence of \( E. coenophiala \) in field tillers was not determined in Jesup MaxQ, Lacefield MaxQ II or Texoma MaxQ II in 2014.

‡ Within a column, means without a common letter differ \( (P < 0.05) \).

### Table 7. Seasonal mean percentage of field tillers of six varieties of tall fescue sampled in 2012 and 2014 across Georgia and Kentucky expressing total ergot alkaloids.

<table>
<thead>
<tr>
<th>Tall fescue variety/Strain of Endophyte</th>
<th>Total ergot alkaloid presence in tillers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
</tr>
<tr>
<td>BarOptima Plus E34</td>
<td>83b†</td>
</tr>
<tr>
<td>IS-FTF 31-UArk9</td>
<td>18c</td>
</tr>
<tr>
<td>Jesup MaxQ†</td>
<td>0d</td>
</tr>
<tr>
<td>Lacefield MaxQ II</td>
<td>0d</td>
</tr>
<tr>
<td>Texoma MaxQ II</td>
<td>0d</td>
</tr>
<tr>
<td>KY31-EI</td>
<td>98a</td>
</tr>
<tr>
<td>KY31-EF</td>
<td>0d</td>
</tr>
<tr>
<td>SEM</td>
<td>2.2</td>
</tr>
<tr>
<td>( P )-value</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

† Within a column, means without a common letter differ \( (P < 0.05) \).

‡ Presence of ergot alkaloid-producing field tillers was not determined in Jesup MaxQ, Lacefield MaxQ II or Texoma MaxQ II in 2014 due to 0% of tillers infected with ergot alkaloids at the initial sampling.

### Total Ergot Alkaloid Content in the Whole Tillers

The locations did not differ or interact with tall fescue variety for [TEA] in the whole tillers of KY 31, BarOptima Plus E34, and IS-FTF 31-UArk9. The data are presented as pooled means across sites. Due to interest in seasonal [TEA] patterns in KY31-EI vs. BarOptima Plus E34, these varieties were sampled more frequently. This resulted in primary sampling dates that included KY31-EI, IS-FTF 31-UArk9, and BarOptima Plus E34, and secondary sampling dates that only included KY31-EI and BarOptima Plus E34 (Fig. 1). However, in 2014, all three cultivars expressing total ergot alkaloids were sampled at the more frequent rate and are therefore presented together (Fig. 2). In 2012, the seasonal mean [TEA] was greater \( (4068 \text{ mg kg}^{-1}, P \leq 0.02) \) in KY31-EI than IS-FTF 31-UArk9 and BarOptima Plus E34 (281 and 280 mg kg\(^{-1}\), respectively). The [TEA] of both IS-FTF 31-UArk9 and BarOptima Plus E34 did on occasion approach the levels identified by Tor-Agbidye et al. (2001) and Craig et al. (2014) as likely to cause fescue toxicosis (Fig. 1, 2 and Table 9). When primary and secondary sampling dates were considered, KY31-EI was greater \( (P < 0.0001) \) than BarOptima Plus E34 at all sampling dates (Fig. 1). Parish et al. (2003) reported mean ergot alkaloids concentrations of 1127 and 822 mg kg\(^{-1}\) for KY 31 at two locations in Georgia during the spring, which is also considerably lower than the current study. Rogers et al. (2011) also reported seasonal [TEA] in KY31-EI grown in Missouri, South Carolina, and Georgia that were considerably lesser \((327– 2411 \text{ mg kg}^{-1})\) than in the current study. Moreover, total ergot alkaloid concentrations at the Missouri site were two-thirds that of the other two locations, likely a result of colder temperatures. This contrasts with the lack of location effect on [TEA] in the current study.

In 2014, the [TEA] pattern was similar to that observed in 2012 among tested varieties (Fig. 2). IS-FTF 31-UArk9
and BarOptima Plus E34 were not different \((P = 0.30; 210 \text{ mg kg}^{-1})\), but less \((P \leq 0.01)\) than KY31-EI \((2758 \text{ mg kg}^{-1})\). Across the season, there was no difference \((P \geq 0.40)\) between BarOptima Plus E34 and IS-FTF 31-UArk9 at any sampling date, whereas KY31-EI contained greater \((P \leq 0.01)\) [TEA] than BarOptima Plus E34 and IS-FTF 31-UArk9 at all sampling dates.

**Ergovaline Content in the Whole Tillers**
The locations did not differ or interact with tall fescue variety for \([E]\) in the whole tillers of KY31-EI, BarOptima Plus E34 and IS-FTF 31-UArk9. Therefore, data are presented as pooled means across sites. As with [TEA], KY31-EI and BarOptima Plus E34 were sampled more frequently than IS-FTF 31-UArk9 in 2012 and are presented as primary and primary + secondary (Fig. 3) sampling dates. However, in 2014, all cultivars were sampled at the same frequency (Fig. 3). The seasonal mean \([E]\) was greater \((P \leq 0.01)\) in KY31-EI \((1841 \text{ mg kg}^{-1})\), than BarOptima Plus E34 and IS-FTF 31-UArk9 \((151 \text{ mg kg}^{-1})\) in 2012. BarOptima Plus E34 and IS-FTF 31-UArk9 were not different \((P = 0.13)\). Similar to 2012, seasonal mean \([E]\) were greater \((P < 0.0001)\) in KY31-EI \((579 \text{ mg kg}^{-1})\) than BarOptima Plus E34 or
IS-FTF 31-UArk9 (80 and 28 mg kg⁻¹, respectively) in 2014. Consistent with [TEA] results, the [E] reported by Rogers et al. (2011) were almost four times lesser than those observed in the current study for KY31-EI. A similar pattern to [TEA] was observed for [E] when primary and secondary sampling dates were included in the analysis for [E] (Fig. 3). KY31-EI was greater (P < 0.0001) [E] than BarOptima Plus E34 on all sampling dates in 2012. In 2012, KY31-EI maintained the greatest [E] (Fig. 3). BarOptima Plus E34 and IS-FTF 31-UArk9 contained the least (P ≤ 0.01) amount of ergovaline at all sampling dates, but were not different (P ≥ 0.41) from each other at any sampling date. Other studies (Belesky et al., 1988; Peters et al., 1992) have shown that [E] in tall fescue are bimodal and peak during the late spring and summer. This pattern was evident in KY31-EI which had greater [E] at Week 6 compared to Week 0; however, as the data was ran as repeated measures, no statistical comparison can be made.

Throughout the 2014 season, there was no difference (P ≥ 0.05) in [E] between BarOptima Plus E34 and IS-FTF 31-UArk9 at any sampling date, though BarOptima Plus E34 tended (P = 0.07) to be greater at Week 13 (Fig. 4). However, KY31-EI contained greater (P ≤ 0.05) concentrations of ergovaline than BarOptima Plus E34 and IS-FTF 31-UArk9 at all sampling dates. Ergovaline concentration of KY31-EI in 2014 was not observed to form a bimodal curve, as was seen in 2012. The pattern was more similar to the findings of Rogers et al. (2011) that reported the highest concentrations of ergovaline in KY31-EI regrowth in September and October instead of May and June.

Total Ergot Alkaloid and Ergovaline Concentrations in Leaf Blade and Sheath

Since [TEA] in the leaf blade differed with year, the data are presented by year. The leaf blades of BarOptima Plus E34, IS-FTF 31-UArk9, and KY31-EI had greater (P ≤ 0.01) seasonal mean [TEA] in 2012 than 2014 (722 vs. 570 mg kg⁻¹; Table 8). As expected, in both years the seasonal mean [TEA] was greater (P < 0.0001) in KY31-EI than BarOptima Plus E34 or IS-FTF 31-UArk9, which were not different (P ≥ 0.95). As a consequence of using composite samples for analysis, due to amount of plant material needed, no statistical analysis was conducted on within season samples. Across the growing season, the [TEA] in the leaf blades ranged from 12 to 421, 50 to 402, and 722 to 2906 mg kg⁻¹, and [E] ranged from 0 to 120, 0 to 50, and 35 to 675 mg kg⁻¹ for BarOptima Plus E34, IS-FTF 31-UArk9, and KY31-EI, respectively.

The [TEA] in the leaf sheath differed with the year (P < 0.0001), so data are presented by year (Table 8). Total ergot alkaloid concentrations were more than 12 times greater (P < 0.0001) in KY31-EI than BarOptima Plus E34 or IS-FTF 34-UArk9 in both years. Additionally, BarOptima Plus E34 and IS-FTF 34-UArk9 were not different (P ≥ 0.85) in either 2012 or 2014. Across the growing season, the [TEA] in the sheaths ranged from 51 to 860, 139 to 1266, and 9120 to 14,705 mg kg⁻¹, and [E] ranged from 0 to 1115, 0 to 300, and 370 to 3012 mg kg⁻¹ for BarOptima Plus E34, IS-FTF 31-UArk9, and KY31-EI, respectively.

Epichloë coenophiala infects the entire plant, colonizing the basal meristem, before its hyphae progress into the intercellular spaces of the leaf sheath (Clay, 1987; White et al., 1993; Christensen et al., 2008). It has been reported that
plants in the reproductive stage have mycelia that are highly concentrated in the base of the plant. Mycelia concentrations decrease upward in the sheath and the ligular zone (Clay, 1987; White et al., 1993; Lane et al., 2000; Takach et al., 2012). Kenyon et al. (2018) found that the segments of plants that are greater than 5 cm from the meristem contained more than 60% less [TEA] compared to the 0- to 5-cm segments. While the current study did not specifically look at vertical distribution, we did observe that the leaf blade contained 73% less [TEA] than the sheath. These findings are congruent with the conclusion of Kenyon et al. (2018), who stated that maintaining stubble height above 5 cm, and thus increasing the proportion of leaf blade consumed, is critical for reducing cases of fescue toxicosis in livestock.

Table 9. Seasonal mean ergovaline (µg kg−1) concentration in the leaf sheath and leaf blade of BarOptima Plus E34, IS-FTF 31-UArk9, and KY 31 varieties of tall fescue sampled during 2012 and 2014 across Georgia and Kentucky.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Leaf Blade</th>
<th>Leaf Sheath</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
<td>2014</td>
</tr>
<tr>
<td>BarOptima Plus E34</td>
<td>148b†</td>
<td>118b</td>
</tr>
<tr>
<td>IS-FTF 31-UArk9</td>
<td>159b</td>
<td>119b</td>
</tr>
<tr>
<td>KY31-EI</td>
<td>1861a</td>
<td>1473a</td>
</tr>
<tr>
<td>SEM</td>
<td>122.2</td>
<td>108.2</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

†Within a column, means without a common letter differ (P < 0.05).

The total [E] in the sheath also differed with the year (P < 0.0001), so data are presented by year (Table 9). Though the magnitude was greater (P ≤ 0.01) in 2012, in both years, sheath [E] were greater in KY31-EI than BarOptima Plus E34 or IS-FTF 34-UArk9, and neither of the latter differed from each other. Both [TEA] and [E] in the sheath were greater in 2012 than 2014 in all three tested tall fescue varieties.

Concentrations of ergovaline were approximately an order of magnitude greater in the sheath samples than the leaf blade samples, regardless of endophyte and variety. Furthermore, [E] were greater in 2012 than 2014 in both leaf blade and sheath samples. This demonstrates the large effect of biotic and abiotic factors that determine total ergot alkaloid and [E] in tall fescue (McCulley et al., 2014).

In conclusion, large variability can occur in tall fescue total ergot alkaloid and ergovaline content over years, within seasons, between cultivars, and within the plant. It confirms the extremely high levels that can be present in wild-type KY 31, and indicates the importance of testing fields for...
total ergot alkaloid and ergovaline content to determine risk to livestock from grazing and hay feeding. It confirms the value of novel endophyte varieties that produce no ergot alkaloids, and demonstrates that while varieties such as BarOptima Plus E34 express consistently lesser levels of ergot alkaloids than KY 31, they can elevate in some circumstances to levels that are greater than considered safe for livestock based on previous studies (Tor-Agbidye et al., 2001; Craig et al., 2014). While the two current tests, [E] and [TEA], provide different predictions of ergot alkaloid severity, they follow the same trends. For some cultivars (i.e., novel-endophyte cultivars), [TEA] may not accurately estimate the effects on livestock. Finally, producers should test existing fields of KY 31 for ergot alkaloid level and consider replanting with a novel endophyte cultivar if ergovaline levels are high (400–750 µg kg⁻¹ and 500–800 µg kg⁻¹ in cattle and sheep, respectively; Tor-Agbidye et al., 2001). The recommended practice for replanting with a novel endophyte cultivar is to spray-kill all existing ergot-incest KY 31 tall fescue forage, as well as seedlings that germinate later from the seed bank in the field, and use an interim crop to further suppress endophyte-infected KY 31 (Roberts and Andrae, 2004). Whereas this practice is highly recommended for replanting tall fescue cultivars with novel endophyte that produce no ergot alkaloids, it is essential to replant with the tall fescue cultivars that produce low levels of ergot alkaloids.

Conflict of Interest
The authors declare that there is no conflict of interest

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References


