

The Effect of Endophytic Fungi on Nematode Populations in Summer-dormant and Summer-active Tall Fescue

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Abstract: Summer-active (continental) and summer-dormant (Mediterranean) tall fescue morphotypes are each adapted to different environmental conditions. Endophyte presence provides plant parasitic nematode resistance, but not with all endophyte strains and cultivar combinations. This study sought to compare effects of four nematode genera on continental and Mediterranean cultivars infected with common toxic or novel endophyte strains. A 6-mon greenhouse study was conducted with continental cultivars, Kentucky 31 (common toxic) and Texoma MaxQ II (novel endophyte) and the Mediterranean cultivar Flecha MaxQ (novel endophyte). Endophyte-free plants of each cultivar were controls. Each cultivar × endophyte combination was randomly assigned to a control, low or high inoculation rate of a mixed nematode culture containing stunt nematodes (*Tylenchorhynchus* spp.), ring nematodes (*Criconebella* spp.), spiral nematodes (*Helicotylenchus* spp.), and lesion nematodes (*Pratylenchus* spp.). Endophyte infection had no effect on nematode population densities. The cultivar × endophyte interaction was significant. Population densities of stunt nematode, spiral nematode, and ring nematodes were higher for Flecha MaxQ than other cultivar × endophyte combinations. Novel endophyte infection enhances suitability of Flecha MaxQ as a nematode host.

Key words: endophyte, host-parasite relationship, lesion nematode (*Pratylenchus* spp.), ring nematode (*Criconebella* spp.), spiral nematode (*Helicotylenchus* spp.), stunt nematode (*Tylenchorhynchus* spp.), summer active, summer dormant, tall fescue.

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumont. = *Lolium arundinaceum* (Schreb.) Darbysh., formerly *Festuca arundinacea* Schreb.] is the dominant cool-season grass in the United States covering over 13 million ha from the Mississippi River to the Atlantic coast and from the Canadian border to South Georgia (Sleper and Buckner, 1995). Areas within this region generally receive annual rainfall of 450 mm or more (Burns and Chamblee, 1979). The annual rainfall requirement of tall fescue limits its adaptation and use west of the Mississippi River. Tall fescue digestibility (Bughrara et al., 1991), crude protein, and yield (Matches, 1979) are high. Yet despite high forage quality, weight gain of steers grazing tall fescue has been reported as low (Hoveland et al., 1983; Read and Camp, 1986; Crawford et al., 1989) as are cow (Schmidt et al., 1983; Danielson et al., 1986; Tucker et al., 1989) and equine performance (Smith et al., 2009). The contradictory aspects of tall fescue, adaptability, good nutritive value, and poor animal performance are solely due to the infection of tall fescue by an endophytic fungus, *Epichloë coenophiala* (Leuchtmann et al., 2014). *Epichloë* species form symbiotic relationships with many cool-season grasses such as tall fescue, and are capable of producing some or all of the bioactive alkaloids, peramine, lolines, ergot alkaloids, and indole-diterpenes that provide the host plant with insect and grazing animal resistance (Clay and Schardl, 2002). Peramine and lolines provide the plant with protection against insect

predation and the ergot alkaloids and the indole-diterpene lolitrem B are best known for causing livestock toxicity, fescue toxicosis, and ryegrass staggers, respectively (Clay and Schardl, 2002).

Consumption of tall fescue containing ergot alkaloids by grazing animals (cattle, sheep, and horses) can create a host of animal disorders collectively referred to as “fescue toxicosis.” Fescue toxicosis causes reduction in serum prolactin levels, reduced animal weight gain and reduction in reproductive efficiency, rough hair coats, and general poor animal performance (Strickland et al., 2012). Early efforts to reduce the detrimental impacts of fescue toxicosis in grazing animals centered on the removal of the endophyte from tall fescue creating endophyte-free varieties, but endophyte-free tall fescue was not as persistent as endophyte-infected tall fescue (Bouton et al., 1993). A later, more successful approach has been the identification of endophytic fungi that do not produce the alkaloids causing livestock toxicity but continue to produce alkaloids for insect predation and improved persistence, which are termed “novel or selected” endophytes (Young et al., 2013; Johnson et al., 2013). These endophytes when placed into tall fescue cultivars result in plants infected with a novel endophyte that is persistent and does not cause tall fescue toxicosis (Bouton et al., 2002; Hopkins et al., 2010). The bulk of these novel endophyte-tall fescue associations are in cultivars that are summer active, originating from temperate climates, and are well adapted to regions in the eastern half of the United States. These temperate varieties are not well adapted to the Southern Great Plains region that is generally hotter and drier, resulting in high tiller mortality (Malinowski and Belesky, 2000). An alternative is tall fescue cultivars selected from Mediterranean climates that possess a summer dormancy trait that allows them to tolerate the adverse Southern Plains summer climate (Malinowski and Belsky, 2000; Malinowski and Pinchak, 2015).

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It has been established that mutualistic endophyte infection improves persistence of summer-active, temperate tall fescue cultivars, but the benefits of endophyte infection of summer-dormant tall fescue is less understood but could impact tolerance to biotic stresses (Malinowski et al., 2012). In a tall fescue stockpile study that compared production and nutritive value of summer-dormant and summer-active cultivars, the cultivar Flecha MaxQ, a summer-dormant morphotype with a novel endophyte, was the only surviving entry after three harvest years. However, it had a reduction in stand count from 99.9% at the beginning of the study to 66% by the study's conclusion (Rogers et al., 2014). In this stockpile study, stand reduction of all cultivars began in the first year. Plant-parasitic nematodes were suspected of having some impact on stand loss and the field was found to have stunt nematodes (*Tylenchorhynchus* spp.) present, though the population was low and unlikely to have been solely responsible for the stand losses. Several types of nematodes are known to affect tall fescue production, with root pruning, reduced forage yield, and plant losses reported (Timper, 2009). Ergot alkaloid-producing endophytes have been shown to reduce population densities of lesion nematode (*Pratylenchus scribneri*) (Panaccione et al., 2006; Bacetty et al., 2009), whereas novel endophyte resistance to lesion nematodes in summer-active cultivars was dependent on the endophyte strain and the tall fescue cultivar (Timper et al., 2005). Comparison of a transgenic nonergot alkaloid-producing strain (deletion of the *dmaW* gene encoding the first step of ergot alkaloid production) to the wild type strain showed endophyte-associated suppression of *P. scribneri* in perennial ryegrass was still evident suggesting other mechanisms might be responsible for the suppression (Panaccione et al., 2006). Reduction in root-knot nematode (*Meloidogyne marylandi*) population densities by ergot alkaloid-producing endophyte infected tall fescue plants has also been reported (Elmi et al., 1999). Each of these studies was conducted with summer-active tall fescue cultivars (Timper et al., 2005; Bacetty et al., 2009) or perennial ryegrass (Panaccione et al., 2006) and may not translate to effects seen with summer-dormant varieties. Therefore, our objective was to evaluate nematode population densities in novel endophyte-infected and endophyte-free summer-dormant and summer-active tall fescue compared to the common toxic endophyte-infected (Kentucky 31+) and endophyte-free summer-active tall fescue.

MATERIALS AND METHODS

Three cultivars of tall fescue were tested in two sequential greenhouse trials in 2011 at the Samuel Roberts Noble Foundation in Ardmore, OK. Cultivars tested were Kentucky 31+ (Ky31+), a summer-active continental type, infected with a ergot alkaloid-producing common

toxic endophyte; Texoma MaxQ II (TMQ+), a summer-active continental type infected with the novel endophyte strain AR584 (Hopkins et al., 2011); and Grasslands Flecha MaxQ (FL+) [Gentos SA (Buenos Aires, Argentina) and AgResearch Grasslands (Palmerston North, New Zealand)] summer-dormant, Mediterranean. Endophyte-free plants of each variety (Ky31-, TMQ-, and FL-) served as controls.

Subsequent to these trials, the FL+ seed stock that was used for this study and a number of field trials was shown to contain a mixture of two different endophytes, the expected AR542 strain and at lower levels AR502 (Young, unpubl. data). When this contamination issue was discovered in the field studies, the present greenhouse study had been terminated. Confirmation of a novel endophyte, lacking the *dmaW* gene that encodes the first step in ergot alkaloid production had been confirmed in FL+ prior to the start of the study and before assigning the plants to a specific replication. With the availability of new markers that can distinguish AR542 and AR502, we were able to go back to the DNA stocks from the original seedling samples and determined that AR502 represented 17% of the endophyte-infected FL+ plants. Unfortunately, no samples were available to test the plants at the end of the trials. AR542 and AR502 represent different endophyte species, *E. coenophiala* and *Epichloë* sp. FaTG-3, respectively, and have slightly different alkaloid profiles. Both endophytes are able to produce peramine and lolines and unable to produce ergot alkaloids, but only AR542 can produce indole-diterpenes such as terpendole C (Takach and Young, 2014). For the purpose of this study, FL+ will be considered endophyte-infected. Whether these two endophytes have different responses to nematodes will need to be further tested.

In February 2011, tall fescue plants were established from seed by placing one seed per cell in a 36-cell tray containing Metro-mix 830 potting soil (Sun Gro Horticulture, Vancouver, British Columbia, Canada). Seventy-two cells, each were established for Ky31+, Ky31-, TMQ+, and TMQ-. One hundred and eight cells, each were established for FL+ and FL- due to lower seed germination. Seeds were germinated in a starter room in the greenhouse facility at 23°C constant temperature and 60% relative humidity. Trays received moisture through a timed misting apparatus that provided uniform water distribution across the trays.

At 7 wk after emergence, a tiller was collected from each plant and tested for endophyte infection using PCR with primers designed to the endophyte genes *tefA* (primers *tef1*-exon1d-1 and *tef1*-exon5-1; Moon et al., 2002) and *dmaW* (primers *dmaW*-F4 and *dmaW*-6R; Takach et al., 2012). The PCR-based screen verified the presence or absence of the endophyte (*tefA* gene marker) and distinguished the novel endophyte from the common toxic endophyte based on the presence or absence of the *dmaW* gene marker (*dmaW* encodes the

first step in ergot alkaloid biosynthesis) and thereby verified the endophyte–plant associations (Takach and Young, 2014; Young et al., 2014).

Following endophyte verification in late April, plants were transferred to 19 × 18 cm, 3.7-liter pots containing a 50:50 by volume mixture of a Renfrow silt loam (Fine, mixed, thermic Udertic Paleustolls) field soil and a Illite calcined clay (Profile Products, Buffalo Creek, IL). Prior to mixing with the clay, the field soil was steam autoclaved at 15 psi and 121°C for 40 min. A liner was placed in the bottom of each pot to prevent the sifting of soil from the pot, the pots were filled with the soil and clay mixture to within 2.5 cm of the top of each pot and then one plant representing one cultivar × endophyte combination was established per pot.

Each trial was a randomized complete block arranged in a factorial design with five replications and a total of 90 individual plants. Within a trial, a pot would contain one plant from one of six tall fescue cultivar × endophyte combinations (Ky31+, Ky31–, FL+, FL–, TMQ+, and TMQ–). On June 3, the cultivar × endophyte combinations were randomly assigned to one of three inoculation rates (control, low, or high) per pot of a mixed nematode culture to determine nematode population effect on carrying capacity of tall fescue cultivar. Nematode inoculum was obtained by collecting soil cores from an established creeping bentgrass (*Agrostis stolonifera*) putting green in Stillwater, OK, known to have high populations of several plant-parasitic nematode species. The soil cores were pooled and the nematodes were extracted using sieving and centrifugal floatation (Ayoub, 1980). Plant-parasitic nematodes were identified to genus and total populations were determined using a compound light microscope. The nematode inoculum contained the migratory ectoparasitic nematodes: stunt (*Tylenchorhynchus* spp.), ring (*Criconemella* spp.), spiral (*Helicotylenchus* spp.), and the migratory endoparasitic lesion nematode (*Pratylenchus* spp.). Nematode inoculation rates are listed in Table 1.

Nematodes were inoculated by pipetting the treatment rate into three holes placed into the soil in the top of each pot and then covering the holes with soil following inoculation. The trials ran for a 6-mon period. Individual pots were watered twice per week and fertilized once per week with 20-10-20 at 100 ppm available N fertilizer solution (Jacks Pro Peat Lite; JR peters, Inc.,

Allentown, PA). Plants were routinely clipped to maintain a 10 cm height to prevent seed formation.

At the termination of the trials, plants were removed from the pots, soil shaken from the roots and bagged, then sent for nematode analysis. Plant roots and shoots were separated and placed into a freezer for future analysis. Roots and shoots were later dried and dry weights recorded. For nematode assays, a 100 cm³ soil subsample was taken from each pot and nematodes were recovered by wet sieving/sugar flotation and identified to genus using a compound microscope.

Nematode population data were analyzed for normality using the PROC UNIVARIATE procedure of SAS, Version 9.3 (SAS Institute, Cary, NC) and the Shapiro–Wilk test for normality. Nematode population data were found to not be from a normally distributed population ($P < 0.01$). Nematode population data were then transformed to log₁₀ values and analyzed using the PROC analysis of variance procedure to test the interaction effects of trial × cultivar, trial × inoculation rate, and trial × endophyte. All interactions were nonsignificant ($P > 0.05$) and data from both trials were combined for further analysis.

Combined, transformed nematode data were then analyzed using the GLIMMIX general linear model procedure of SAS (SAS Institute). Trial and replication were considered to be random effects and cultivar, endophyte, inoculation rate, and their interactions fixed effects. The LSMEANS procedure was used with means adjusted by the Tukey–Kramer method to detect differences in fixed effects and interactions. Differences of LSMEANS were declared significant at $P \leq 0.05$. Non-transformed means are reported in tables and discussion. Data for plant root and shoot mass were analyzed using the same procedures as was used for the nematode data.

RESULTS

Lesion nematode population density: Lesion nematode population numbers were not affected ($P > 0.05$) by cultivar, nematode treatment level, endophyte infection, or their interactions (Table 2). Lesion nematode results are difficult to interpret as they are an endoparasitic nematode and move from the soil into the plant roots where they cause root lesions, root stunting,

TABLE 1. Nematode culture inoculation rates per pot that were applied to soil containing summer-dormant or summer-active tall fescue cultivars either infected or noninfected with an endophytic fungus.

Inoculation rate	Stunt nematodes (<i>Tylenchorhynchus</i> spp.)	Ring nematodes (<i>Criconemella</i> spp.)	Spiral nematodes (<i>Helicotylenchus</i> spp.)	Lesion nematodes (<i>Pratylenchus</i> spp.)
	Nematode culture populations per pot			
Control	0	0	0	0
Low	90	250	75	20
High	270	800	225	55

TABLE 2. Analysis of variance (Type III test for fixed effects) for log₁₀ transformed nematode population density counts from soil of pots containing tall fescue cultivars either infected or noninfected with an endophytic fungus and one of three nematode inoculation rates.

Effect	Spiral nematode (<i>Helicotylenchus</i> spp.)	Stunt nematode (<i>Tylenchorhynchus</i> spp.)	Lesion nematode (<i>Pratylenchus</i> spp.)	Ring nematode (<i>Criconebella</i> spp.)
Cultivar (C)	**	NS	NS	***
Inoculation rate (IR)	***	***	NS	***
Endophyte (E)	NS	NS	NS	NS
C × IR	NS	NS	NS	***
C × E	**	***	NS	***
E × IR	NS	NS	NS	NS
C × IR × E	NS	NS	NS	***

Data are combined from two trials with five replications per trial.

** $P \leq 0.05$ level of significance.

*** $P \leq 0.01$ level of significance.

and dieback, which results in a reduction in plant growth. Some visible root lesions on plant roots were observed but no extraction of lesion nematodes from the roots was done. A repeat of this study should include root extraction for lesion nematodes.

Spiral and stunt nematode population densities: Nematode inoculation rate was significant ($P < 0.05$) for spiral nematode and stunt nematode (Table 2). The spiral nematode differences between the high inoculation rate (243/100 cm³ soil) and low inoculation rate (160/100 cm³ soil) were not significant ($P > 0.05$) but both inoculation rates were greater ($P < 0.05$) than the control (34/100 cm³ soil). The response for stunt nematodes was similar, the high inoculation rate (68/100 cm³ soil) and low inoculation rate (54/100 cm³ soil) were not significantly different ($P > 0.05$) but both were greater ($P < 0.05$) than the control (7/100 cm³ soil). The cultivar × inoculation rate interaction was significant ($P < 0.05$) only for ring nematode (Table 2).

As a main effect, endophyte presence or absence had no effect on spiral and stunt nematode population densities. However, the cultivar × endophyte interaction was significant ($P < 0.05$) for both spiral and stunt nematodes (Table 2). FL+ had higher spiral and stunt nematode population densities than the other cultivar × endophyte combinations (Table 3). The remaining

TABLE 3. Effect of tall fescue cultivar and endophyte infection status on mean spiral nematode (*Helicotylenchus* spp.) and stunt nematode (*Tylenchorhynchus* spp.) population counts per 100 cm³ soil volume.

Cultivar × endophyte	Spiral nematodes per 100 cm ³ soil	Stunt nematodes per 100 cm ³ soil
FL+	330 a ^{ab}	91 a
FL-	147 b	40 b
Ky31+	55 b	20 b
Ky31-	174 b	40 b
TMQ+	84 b	35 b
TMQ-	80 b	32 b

Means are combined across two trials with five replications per trial.

^a Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

^b Data were transformed [log₁₀] prior to analysis. Nontransformed means are shown in the table.

cultivar × endophyte combinations (FL-, Ky31+, Ky31-, TMQ+, TMQ-) were similar in populations densities of spiral and stunt nematodes.

Ring nematode population density: Endophyte presence or absence had no effect ($P < 0.05$) on population density response of ring nematode as was also seen for spiral, stunt, and lesion nematodes (Table 2). Ring nematode population density was significantly affected ($P < 0.05$) by the three-way interaction of cultivar × inoculation rate × endophyte infection (Table 2). FL+ at low (250/100 cm³ soil) and high (800/100 cm³ soil) nematode inoculation rates were similar ($P > 0.05$) in ring nematode population density (Table 4). Ring nematode population density for FL+ at the low (250/100 cm³ soil) inoculation rate was higher ($P < 0.05$) than TMQ-, TMQ+, Ky31-, Ky31+, regardless of their

TABLE 4. The effect of tall fescue cultivar, endophytic infection, and ring nematode inoculation rate on ring nematode (*Criconebella* spp.) population density means per 100 cm³ soil.

Cultivar	Endophyte status	Inoculation rate ^a	Population density
FL+	Present	Low	1026 a ^{bc}
FL+	Present	High	606 ab
FL-	Absent	High	311 bc
TMQ-	Absent	Low	291 bc
Ky31-	Absent	High	246 bc
TMQ-	Absent	High	236 bc
FL-	Absent	Low	162 bc
TMQ+	Present	High	159 bc
Ky31-	Absent	Low	64 c
TMQ+	Present	Low	34 c
FL+	Present	Control	31 c
Ky31-	Absent	Control	27 c
Ky31+	Present	Low	14 c
TMQ+	Present	Control	13 c
TMQ-	Absent	Control	9 c
Ky31+	Present	High	6 c
Ky31+	Present	Control	3 c
FL-	Absent	Control	2 c

Data are combined from two trials with five replications per trial.

^a Ring nematode (*Criconebella* spp.) inoculation rates: control = 0 per pot; low = 250 per pot; high = 800 per pot.

^b Least squares means followed by the same letters are not different for population density ($P \leq 0.05$).

^c Data were transformed [log₁₀] prior to analysis. Nontransformed means are shown in the table.

TABLE 5. Analysis of variance (Type III test for fixed effects) for \log_{10} transformed nematode reproductive factor (final population density/initial population density) from tall fescue cultivars either infected or noninfected with an endophytic fungi and one of three nematode inoculation rates.

Effect	Spiral nematode (<i>Helicotylenchus</i> spp.)	Stunt nematode (<i>Tylenchorhynchus</i> spp.)	Lesion nematode (<i>Pratylenchus</i> spp.)	Ring nematode (<i>Criconebella</i> spp.)
Cultivar (C)	***	NS	NS	***
Inoculation rate (IR)	***	***	NS	***
Endophyte (E)	NS	NS	NS	NS
C × IR	***	NS	NS	***
C × E	***	NS	NS	**
E × IR	NS	NS	**	NS
C × IR × E	NS	NS	NS	NS

Data are combined from two trials with five replications per trial.

** $P \leq 0.05$ level of significance.

*** $P \leq 0.01$ level of significance.

inoculation rate (Table 4). No differences are noted in ring nematode population density numbers between TMQ+ and TMQ- implying that the novel endophyte strain AR584 had no effect on ring nematode population density of TMQ regardless of inoculation rate. No differences ($P > 0.05$) were noted across control inoculation rates (Table 4).

Reproductive factor: A reproductive factor (R_f) for each nematode species within each cultivar × endophyte combination in the study was calculated by taking the final population of each species and dividing by the initial nematode species population. R_f was then used to categorize the suitability of the tall fescue cultivars as a nematode host by a rating system (Ferris et al., 1993) of excellent host ($R_f > 10$), good host ($10 > R_f > 1$), maintenance host ($R_f \sim 1$) and poor or nonhost ($R_f < 1$).

Nematode inoculation rate was significant ($P < 0.05$) for spiral nematode, stunt nematode, and ring nematode (Table 5). R_f factor was less than 1 for the stunt control, low, and high inoculation rates. A cultivar × inoculation rate was significant ($P < 0.05$) for spiral nematode and ring nematode (Table 5). FL low inoculation rate had an R_f of 3.8, significantly greater ($P < 0.05$) than other cultivar × inoculation rate combinations. This same effect was true for ring nematode.

FL at low inoculation rate had an R_f of 2.38 for ring nematode compared to an $R_f < 1$ for all other cultivar × inoculation rate combinations.

A cultivar × endophyte interaction had a significant ($P < 0.05$) effect on R_f for spiral nematode and ring nematode. The cultivar FL+ had a 1.62 R_f rating and would be characterized as a good host for ring nematodes compared to other cultivar × endophyte combinations (Table 6). FL+ (R_f 2.42) is a good spiral nematode host ($P < 0.05$) compared to Ky31+, TMQ+, and TMQ- all of which are rated as poor hosts for spiral nematodes (Table 6). FL+, FL-, and Ky31- have a similar ($P > 0.05$) R_f and would be characterized as a poor host for spiral nematode.

Root and vegetative mass: Cultivar and endophyte presence or absence had a significant effect ($P < 0.05$) on tall fescue root mass (Table 7). Other main effects and their interactions were not significant. The cultivar FL had greater mean root mass than TMQ or Ky31 (Fig. 1). Endophyte absence significantly increased ($P < 0.05$) root mass (Fig. 1).

Results of vegetative mass production were similar to root mass as FL produced more vegetative mass than TMQ or Ky31 (Fig. 2). In a field study, FL produced similar forage yields as TMQ and Ky31 (Rogers et al.,

TABLE 6. Tall fescue endophyte by cultivar effect on the reproductive factor (population final/population initial) means of four nematode species per 100 cm³ soil.

Cultivar	Reproductive factor ^a			
	Stunt nematode (<i>Tylenchorhynchus</i> spp.)	Ring nematode (<i>Criconebella</i> spp.)	Spiral nematode (<i>Helicotylenchus</i> spp.)	Lesion nematode (<i>Pratylenchus</i> spp.)
FL+	0.57 ^b	1.62 a ^c	2.42 a	0.02
FL-	0.28	0.34 b	1.18 ab	0.02
Ky31+	0.15	0.02 b	0.41 b	0.01
Ky31-	0.30	0.19 b	1.03 ab	0.07
TMQ+	0.20	0.11 b	0.67 b	0.06
TMQ-	0.17	0.49 b	0.61 b	0.05

Data are combined from two trials with five replications per trial.

^a Reproductive factor rating system (R_f) indicate excellent host ($R_f > 10$), good host ($10 > R_f > 1$), maintenance host ($R_f \sim 1$) and poor or non-host ($R_f < 1$) (Ferris et al., 1993).

^b Data were transformed [\log_{10}] prior to analysis. Nontransformed data are shown in the table.

^c Means within column followed by the same letter are not different ($P \leq 0.05$).

TABLE 7. Analysis of variance (Type II test for fixed effects) for vegetative and root mass from tall fescue cultivars either infected or noninfected with an endophytic fungi and three nematode inoculation rates.

Effect	Vegetative mass	Root mass
Cultivar (C)	***	***
Inoculation rate (IR)	NS	NS
Endophyte (E)	NS	**
C × IR	***	NS
C × E	NS	NS
E × IR	NS	NS
C × IR × E	NS	NS

Data are combined from two trials with five replications per trial.
 ** $P \leq 0.05$ level of significance.
 *** $P \leq 0.05$ level of significance.

2014), which supported these greenhouse vegetative mass results. A tall fescue cultivar × nematode inoculation rate interaction was significant for vegetative mass (Table 7). Within cultivars, vegetative mass was not effected by nematode inoculation rate (Fig. 3) though difference between cultivars and inoculation rates are noted.

DISCUSSION

The presence or absence of the tall fescue endophytic fungi *E. coenophiala* had no effect on nematode population densities in this greenhouse study. In a tall fescue study that included the MaxQ (AR524) novel endophyte, no endophyte status effect was shown for ring nematode reproduction when pots were inoculated with 1,000 adults and juvenile ring nematodes (Nyczepir, 2011). In addition, there was no tall fescue cultivar effect on ring nematode reproduction (Nyczepir, 2011). Our results found a cultivar by endophyte interaction influenced both spiral nematode and stunt nematode population densities. The novel endophyte-infected cultivar FL+ had significantly higher spiral nematode and stunt nematode population densities than FL-, Ky31+, Ky31-, TMQ+, and TMQ-, which

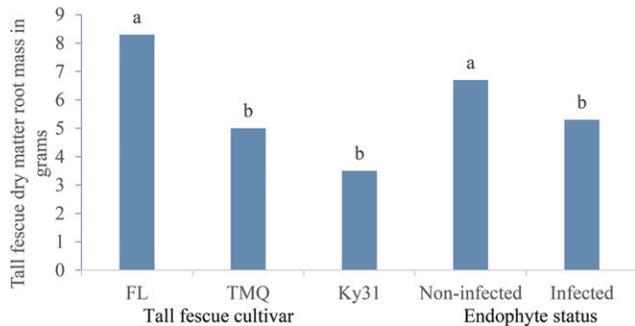


FIG. 1. The main effects of tall fescue cultivar and endophyte (noninfected or infected) on tall fescue root mass (grams) means across two trials with five replications per trial. Means within tall fescue cultivar or endophyte status with the same letter are not significantly different ($P \leq 0.05$).

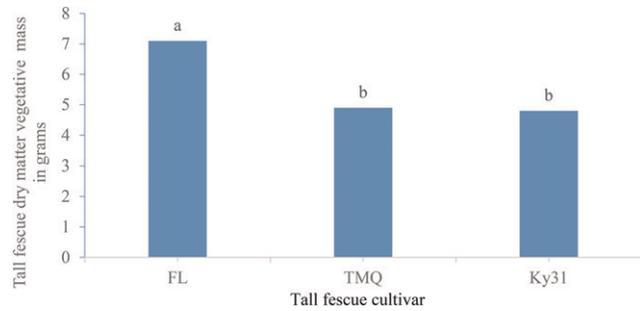


FIG. 2. The main effect of tall fescue cultivar on tall fescue vegetative mass means (grams) across two trials with five replications per trial. Tall fescue cultivar column means with the same letter are not significantly different ($P \leq 0.05$).

contained similar nematode population densities. Cultivar × endophyte × nematode inoculation rate significantly affected ring nematode population densities. At the low inoculation rate, FL+ ring nematode was similar to FL+ at the high inoculation rate. However, FL+ ring nematode was greater than the remaining cultivar × endophyte × inoculation rates. FL+ had a higher *Rf* rating for ring nematode than other cultivar by endophyte combinations. FL+, FL-, and Ky31- had a similar *Rf* rating for spiral nematode, but FL+ *Rf* was higher than Ky31+, TMQ+, and TMQ-. From these data, we would then conclude that FL+ novel endophyte-infected cultivar is a more suitable host for ring nematode, spiral nematode, and stunt nematode. In earlier work with lesion nematode, the novel endophyte strain AR542 infecting the tall fescue cultivar Jesup did not confer lesion nematode resistance (Timper et al., 2005).

Within the continental tall fescue types (Ky31+, Ky31-, TMQ+, TMQ-), there were no differences in lesion, stunt, or ring nematode population densities regardless of endophyte infection. *Rf* data indicate Ky31+, TMQ+, and TMQ- are a poor host for stunt, ring, and spiral nematodes. Ky31- is a poor host for stunt and ring nematodes, but a good host for spiral nematode. In previous work, the wild type endophyte has

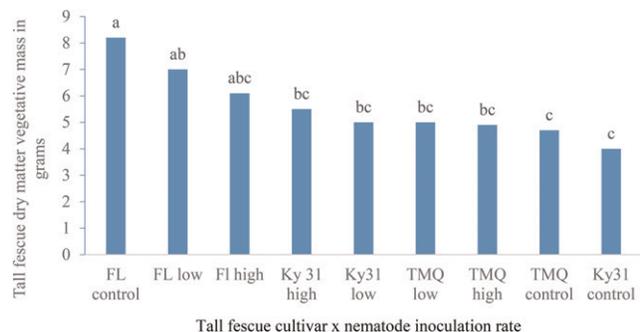


FIG. 3. The tall fescue cultivar × nematode inoculation rate on tall fescue vegetative mass means (grams) across two trials with five replications per trial. Column means with the same letter are not significantly different ($P \leq 0.05$).

been shown to suppress lesion nematode populations, (Timper et al., 2005; Panaccione et al., 2006; Bacetty et al., 2009; Timper and Bouton 2012), whereas Kimmons et al. (1990) found no difference in spiral nematode numbers in pots containing either endophyte-free or endophyte-infected (toxic) tall fescue. Results from this study support this previous study, but we also show no difference between TMQ+ (AR584 novel endophyte) and TMQ–.

An explanation to differences in root weights could be attributed to the origins of the cultivars. FL originated for the Mediterranean basin region and is summer dormant while the other cultivars are of European continental origin and are summer active or summer semidormant. The summer dormancy trait may impart emphasis on root growth and development as part of its survival mechanism to avoid and persist through periods of high temperature and drought.

Based on these data, the novel endophyte AR584 is conveying to the tall fescue cultivar TMQ similar nematode suppression of spiral, stunt, and ring nematodes as Ky31+ infected with a common toxic endophyte. In addition, the nematode host suitability appears to be enhanced for endophyte-infected FL+. This effect may be due to the plant genotype effect of suppressing endophyte growth or the endophyte may suppress the plant's natural ability to suppress nematode populations. Meyer et al. (2013) reported that endophyte status of Jesup MaxQ did not appear to be the primary factor in root-knot nematode (*Meloidogyne incognita*) suppression, but rather root and shoot compounds produced by the cultivar are nematostatic or nematotoxic to root-knot second stage juveniles. This could further help explain the cultivar by endophyte status effect seen for FL. Spiral, stunt, and ring nematode population densities were lower for FL– compared to FL+. FL– could be producing similar root and shoot exudates that are causing nematode suppression compared to FL+, which will need further investigation.

The role of *E. coenophiala* in its association with continental type tall fescue is understood and well documented whereas the role of endophyte associations in Mediterranean tall fescue is not (Malinowski et al., 2012). The main advantage of endophyte infection conferred to continental tall fescue is improved plant persistence during hot, dry summers. Since summer-dormant tall fescue types simply avoid these periods by going dormant, endophyte infection may not provide any additional drought tolerance and could possibly interfere with the plants ability to produce nematode suppressive exudates. Results of this study would indicate that interactions of tall fescue plant genotype × novel endophyte effects need additional study as to the suppression affect they may confer to plant parasitic nematodes and the impact on plant persistence and biomass production.

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