Infection of Tall Fescue Cultivars with Non-toxic Endophytes

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For tall fescue in the southeastern USA, long term persistence, competitiveness, yield, and summer survival are directly related to the presence of a fungal endophyte (*Neotyphodium coenophialum*; formerly called *Acremonium coenophialum*) living in the fescue plant. However, cattle grazing forage from the predominant USA tall fescue cultivars infected with their naturally occurring endophyte strains (E+) suffer from a condition called "*Fescue Toxicosis*". Occurrence of fescue toxicosis is associated with the presence of this endophytic fungus.

Endophyte Effects on Summer Survival

Summer drought results in the greatest loss of tall fescue stands in the southeast. Table 1 dramatically shows the value of infection with the *N. coenophialum* endophyte for survival in a very hot, dry summer in Georgia (Bouton et al. 1993a). Literally, the endophyte-free (E-) versions of all cultivars in this particular experiment died completely while the E+ versions maintained good stands.

Another stress factor for tall fescue pastures is encroachment by bermudagrass. This aggressive, warm season grass has been observed to reduce the stands of both E+ and E- tall fescue especially when combined with grazing in hot, dry summers. To better assess the effect of both bermudagrass competition and grazing, E+ and E- tall fescue cultivars were tested under grazing or hay clipping management after their establishment in tilled soil or bermudagrass sod seeded conditions (Table 2). The positive effect of endophyte infection on plant survival was seen by the better survival of E+ 'Georgia 5' compared to E- 'AU Triumph' in all testing conditions. Grazing, when combined with bermudagrass competition, created the greatest stress for tall fescue survival. For future studies to rapidly assess stand persistence of either E+ or E- tall fescue cultivars, plots can be established in bermudagrass sods and grazed. Besides defining a worse-case but real-world scenario in the southeast region, this type of screening procedure allows cultivars to be assessed more quickly and efficiently.

Endophyte Effects on Grazing Animals

The fescue toxicosis condition is a result of ingestion of ergot alkaloids derived from the endophyte association (Hill et al., 1994) that causes poor weight gain and reproduction in afflicted animals (Hoveland et al., 1983; Hoveland et al. 1997). Typically, animal weight gains during spring and early summer on E+ pastures are much lower than those on E- pastures (Table 3).

Infection of Tall Fescue Cultivars with Non-toxic Endophyte Strains

The toxicity of E+ tall fescue presents livestock producers with a dilemma of whether to grow current E+ cultivars for stand persistence but risk reduced animal performance due to the inherent toxins. There are two potential approaches open to overcome this problem; management options to reduce toxicity in E+ pastures (e.g. inter-planting with clovers, etc.) or developing persistent E- cultivars or E+ cultivars with reduced or nil production of toxic alkaloids. For the cultivar development option, the main objectives are as follows: 1) reduce the levels of alkaloid production in E+ cultivars through genetic selection, or 2) remove native toxic strains from elite cultivars and re-infect them with nontoxic endophyte strains. Since we were able to obtain non-toxic *N. coenophialum* strains from Dr. Garry Latch (AgResearch Grasslands, Palmerston North, New Zealand), we felt the second option held a great deal of promise for us.

We had also previously released two cultivars for use in the southeast. Georgia 5 shown in Table 2 above was developed as an E+ cultivar for the coastal plain region (Bouton et al. 1993b). The Jesup cultivar (Bouton et al. 1997) was developed as a E- cultivar for use in the main fescue growing regions due mainly to its excellent animal performance results (Table 3). However, as is the case with all tall fescue cultivars, these two show better summer survival when grown E+ (Bouton et al. 1993a). Therefore, the adaptation and excellent performance of Georgia 5 and Jesup, especially when infected with endophytes, made them good candidates for re-infection with non-toxic endophytes.

Objective

The objective of this paper is to report our testing results for summer survival and animal performance of Jesup and Georgia 5 after re-infection with a non-toxic endophyte strain.

Materials and Methods

The non-toxic *N. coenophialum* AgResearch strain used in these experiments was AR542. Re-infection of this strain into a random sample of several hundred seedlings of E- Jesup and E- Georgia 5 tall fescue was obtained using procedures from Latch and Christensen (1985). Seed of these re-infected plants were then increased in isolation for two generations using extra plants of Jesup or Georgia 5 as additional pollen parents.

Jesup (AR542) and Georgia 5 (AR542) were initially tested against E+ and E- Jesup in lamb grazing trials during March - June and October - December, 1998-99 at Eatonton, GA. Paddock size was 0.2 acre. There were 2 reps of each entry and stocking rate was 4 (1998) or 5 (1999) 50 lb animals per paddock. Every 2 weeks, weight gain and serum prolactin in the animals and available yield and ergot alkaloid concentration in the forage were recorded. These same entries were tested in a beef steer grazing trial during 1999 at the same location. Paddock size was 2 acres and there were 2 reps of each entry. Depending on the season, stocking rate was two or three 550 lb animals per paddock. Every two weeks weight gain and serum prolactin levels in the animals and yield and ergot alkaloid concentration in the forage were recorded.

In a separate experiment, summer survival was assessed by sowing seed of all three versions of Jesup and Georgia 5 (E+, E-, and AR542) into bermudagrass sod in October 1997 and grazing with beef cattle from April until November in each of the next two years. Stand

assessments were made on April 1998 (initial), March 1999, and December 1999.

Results of Animal Studies

Cultivars with the AR542 strain produced none of the toxic ergot alkaloids in their forage and lambs grazing them had approximately 50% more weight gain than lambs on E+ forage and gain similar to lambs grazing E- forage (Table 4). An additional measure of toxicosis was found in blood samples for prolactin concentration. Blood prolactin was dramatically reduced in animals grazing E+ forage when compared to those on E- forage (Table 4). The lambs consuming forage with the AR542 strain showed blood prolactin levels similar to lambs on E- pastures further indicating a lack of toxicity. Similar results were obtained for lambs during the fall grazing period in both years (data not shown).

Beef cattle paddocks were newly planted during the fall of 1998. Due to dry weather and limited annual ryegrass contamination, we only conducted short-term grazing for animal performance during the late spring of 1999. A more normal grazing was then conducted during the fall of the year. Results indicate good performance of E- and the AR542 strain in providing better animal performance (Table 5) and indicate the value of removing the toxins from tall fescue in providing better and more economical beef gain. Differences between entries for serum prolactin and forage ergot concentration in these beef performance trials (data not shown) were similar to those found in the lamb study reported in Table 4.

Results of Summer Survival Studies

Grazing, when combined with bermudagrass competition, was found in a previous studies to create the greatest summer stress on tall fescue (Table 2). Besides defining a real world but worse case screening environment, it allows E+ and E- cultivars to be separated more quickly and efficiently for summer survival. When screened in this manner, Georgia 5 and Jesup with AR542 were found to possess better summer survival than their E- versions and possess survival equal to their E+ versions after one year (Table 6). Although stands of all entries were further depleted during a second summer, the E+ and AR542 versions of both cultivars were all found to be superior to their E- checks; however, only Jesup (AR542) was still found to be equivalent to its E+ check in stand survival (Table 6).

Conclusions

These studies indicate that re-infection of non-toxic endophyte strains into elite cultivars is a good strategy to reduce toxicity and provide better animal performance and summer survival in tall fescue pastures. Our immediate plans are to repeat these current experiments and expand testing into other locations. Finally, the AR542 strain shown in Tables 4-6 will be marketed by Pennington Seed, Madison, GA, under the trade-marked name of “MaxQ” beginning in the fall of 2000. Initially, MaxQ will be sold in the Jesup and Georgia 5 cultivars and will be positioned for the professional farmer capable of providing the best management as outlined in Pennington’s MaxQ User Guide.

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Table 1. Stand percentage of tall fescue differing in fungal endophyte (*Neotyphodium coenophialum*) infection. Data pooled across 4 cultivars (from Bouton et al. 1993a).

Endophyte†	May	September
	---------%---------	
E+	75*	91**
E-	67	0

†E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar;
E- = Nil or *Neotyphodium* free cultivar.
*, ** Significantly different at the $p<0.05$ and 0.01, respectively.

Table 2. Effect of two different management (clipping for hay or grazing) combined with two establishment conditions (seeded into tilled soil and sod seeded into bermudagrass) on stand percentage of tall fescue cultivars two years after establishment (J.H. Bouton and R.N. Gates, 1998, Unpublished).

Cultivar	Endophyte†	Clip-Till	Graze-Till	Clip-Sod	Graze-Sod
		----------%----------			
Georgia 5	E+	93.6	78.2	91.2	59.2
AU Triumph	E-	83.4	50.6	34.8	20.0
LSD ($p<0.05$)		NS	24.9	15.9	22.9

†E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar;
E- = Nil or *Neotyphodium* free cultivar.

Table 3. Beef steer performance as affected by endophyte infection in 'Jesup' tall fescue during 3 spring grazing seasons at Eatonton, GA (Hoveland et al. 1997).

Cultivar	Endophyte†	Average Daily Gain	Gain per Steer	Gain per Acre
		----------lb----------		
Jesup	E+	0.8 b‡	63 b	89 b
Jesup	E-	2.3 a	176 a	256 a

†E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar; E- = Nil or *Neotyphodium* free cultivar.
‡Means within a column with different letters are significantly different ($p<0.05$)

Table 4. Results of a lamb grazing trial during the spring grazing period for 1998 and 1999 at Eatonton, GA.

		Forage		Animals		
Cultivar	Endophyte Strain†	Available	Ergot Yield	Average Alkaloids	Blood Daily Gain	Prolactin
		lbs/acre	*ppm*	*lbs/day*		*ng/ml*
Jesup	E+	1780	3.6 a‡	0.19	4 b‡	
Jesup	E-	1496	0 b	0.27	188 a	
Jesup	AR542	1837	0 b	0.29	217 a	
Georgia 5	AR542	1939	0 b	0.30	178 a	
LSD (<0.05)		NS	------	0.04	------	

†E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar; E- = Nil or *Neotyphodium* free cultivar; AR542 = Non-toxic AgResearch *Neotyphodium* strain not producing ergot alkaloids.
‡Means within column followed by the same letter are not significantly different ($p<0.05$) by LSD comparisons calculated on data subjected to square root transformation.

Table 5. Beef steer performance trial at Eatonton, GA during the spring and fall grazing periods for 1999.

Cultivar	Endophyte Strain §	Spring † Average Daily Gain	Spring † Gain per Acre	Fall ‡ Average Daily Gain	Fall ‡ Gain per Acre
		lbs		lbs	
Jesup	E+	1.7	83	1.9	169
Jesup	E-	1.9	93	2.7	235
Jesup	AR542	2.5	123	2.6	223
Georgia 5	AR542	2.7	133	2.7	237
LSD (<0.05)		0.7	36	0.5	42

† Stocking rate = 1.0 steer per acre; grazing period = 49 days.
‡ Stocking rate = 1.5 steers per acre; grazing period = 58 days.
§ E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar; E- = Nil or *Neotyphodium* free cultivar; AR542 = Non-toxic AgResearch *Neotyphodium* strain not producing ergot alkaloids.

Table 6. Summer survival of two tall fescue cultivars with different *Neotyphodium coenophialum* endophyte strains. All cultivars planted into bermudagrass and grazed during April - November, 1998-99 at Eatonton, GA.

Cultivar	Endophyte Strain †	Sampling Date 5-7-98	5-18-99	12-7-99
		% occupancy ‡		
Jesup	E+	98.2	74.4	47.5
Jesup	E-	95.5	29.1	10.7
Jesup	AR542	93.7	80.0	42.0
Georgia 5	E+	95.7	66.0	41.7
Georgia 5	E-	94.8	22.2	9.5
Georgia 5	AR542	93.3	62.5	24.7
LSD (p<0.05)		NS	15.9	10.3

† E+ = Contains *Neotyphodium* strain(s) naturally occurring in the cultivar; E- = Nil or *Neotyphodium* free check; AR542= Non-toxic AgResearch *Neotyphodium* strain not producing ergot alkaloids.
‡ Percentage of the 4 inch increments of the original 10 foot long drilled rows in the sward occupied by tall fescue tillers.

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