History of Endophytic Toxicosis and Novel Variety Solutions

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Abstract. Associations of *Epichloe* endophytes with tall fescue [*Lolium arundinaceum* (Schreb). Darbush.] and perennial ryegrass (*Lolium perenne* L.) form the basis for the known science and ecology of mutualistic, fungal endophytes, and especially their impact on livestock. In the USA, millions of acres are endophyte infected today due mainly to planting 'Kentucky 31' to reduce soil erosion. Health problems emerged later among animals grazing the grass. These toxicity symptoms were listed under the general term 'Fescue Toxicosis'. Research demonstrated that ergot alkaloids produced by the mutualism were mainly responsible. Developing endophyte-free versions of major varieties reduced host grass persistence and performance. Farmers seemed stuck with a classic dilemma: animal health or pasture persistence? However, research found that naturally occurring, nontoxic endophyte strains (e.g., "novel") could be isolated and reinfected into elite grass varieties. Novel varieties restored persistence with none of the toxic alkaloids. The development and commercialization of the first novel endophyte tall fescue variety, Jesup MaxQ, is reported here. Other varieties were developed, and the seed trade now recognizes 3 variety types: toxic, endophyte-free, or novel. However, seed sales of novels are still comparatively low, and The Alliance for Grassland Renewal was formed to advance their use.

Introduction

"I'm like Loki in Nordic mythology: one day I'll be a woman and the next day a snake". Yung Lean

The history of endophytic fungi in grasses is compelling; even mythological: "once upon a time, there was a creature that lived inside perennial ryegrass and tall fescue and gave these grasses special powers for both good and bad". For tall fescue in the USA, this history begins mainly with the release and widespread planting of Kentucky 31 that is recorded extensively in the book The Wonder Grass (Ball et al. 2019). This reference is summarized in the following sections.

A Farmer, A Hillside in Kentucky, and a University of Kentucky Agronomist

A mountain farmer, William M. Suiter, in 1887, purchased three tracts of land in Menifee County, Kentucky. On one of these tracts, Mr. Suiter noticed an unusual grass growing in patches on a hillside. He harvested seed from these patches and used it to thicken the entire hillside. It eventually became the dominant grass on his property. He sold small amounts of seed to a few neighbors, but in later years when the demand for "Suiter's Grass" became high, he put more focus on seed production. In 1931, Dr. Ian Fergus, University of Kentucky (UK) agronomist, traveled to Menifee County, where he saw the grass and took about a pound of seed back with him to the UK campus and established it in evaluation plots. Due to the growing popularity of Suiters Grass, Dr. Fergus, with encouragement from his colleague William Johnstone, in 1942 released the seed from his Lexington plots as Kentucky 31 (named for 1931 collection date). Tall fescue is now estimated to be established in 30-40 million USA acres.

Problems Begin: "Fescue Toxicosis"

The main driver for the expanded acreage of tall fescue in general, and Kentucky 31 in particular, was for its use in soil erosion control. After obtaining productive stands, the obvious economic use was as general pasture. Eventually, forage-livestock farmers began reporting animal problems such as "fescue foot" and "summer slump" with these now classified under the general heading of "fescue toxicosis".

Research: It's the fungus

It has been over 40 years since pioneering work by C.W. Bacon, J.D. Robbins, and J.K. Porter reported that fungal endophytes were the main cause of livestock toxicosis in a mutualistic relationship with tall fescue. Reports by L.R. Fletcher and I.C. Harvey in New Zealand documented a similar association with a closely related endophyte in perennial ryegrass. Multidiscipline research reported on all aspects of these important grass-fungal mutualisms. Intensive research results were presented at many regional, national, and international meetings that continue to this day. The main science paper citations are also recorded in The Wonder Grass (Ball et al. 2019). The mutualist, endophytic fungus that grows between the tall fescue plant cells is classified as *Epichloë coenophiala*. Ergot alkaloids produced by the mutualism are the main cause of fescue toxicities.

Remove the Fungus?

Unfortunately, removing the toxic fungal endophyte (E+) from the seed also reduced stand survival. Further research showed multiple reasons why toxic endophytes mutualisms make tall fescue more persistent and ecologically "fit" and act in the plant like a "super gene". Reduced grazing due to toxic ergot alkaloids during late spring allows better forage accumulation leading to an "insulation" effect and better summer survival. All alkaloids produced are not bad (peramine has anti-insect properties). Toxic mutualisms produce plant growth hormones that result in better plant tillering and yield and also increase leaf rolling leading for better drought tolerance.

Classic Dilemma

Farmers were stuck with a classic dilemma: Animal health or pasture persistence? Strategies available were 1) mitigation of toxicities by inter-planting clovers, feeding other forages, removing animals during worst toxic times, removing seedheads, etc.; or 2) simply better (expensive) management of endophyte-free varieties including long rest periods. Most farmers did not practice either strategy but were less inclined to plant endophyte-free varieties. Most simply lived with the problem.

A Novel Solution

Development of the first nontoxic (now called "novel") endophyte tall fescue variety, Jesup MaxQ, is recorded in The Wonder Grass, (Ball et al. 2019). Since it began in the International Grassland Congress (IGC) in Nice, France, the story is pertinent to the current IGC and quoted as follows:

"The main "tipping point event" underlying the development of Jesup MaxQ occurred at the 1989 International Grassland Congress in Nice, France. It was a chance and serendipitous meeting and discussion at that Congress between two scientists from normally disparate disciplines and even different parts of the world. These scientists were G.C.M. Latch, who worked with AgResearch in New Zealand, and J.H. Bouton, a forage breeder at the University of Georgia (UGA)".

Dr. Latch was reporting on his research with deploying novel endophytes into perennial ryegrass cultivars. During discussion, Latch learned that the UGA varieties, Jesup and Georgia 5, were more persistent when infected with its toxic endophyte. Since AgResearch had novel tall fescue endophyte strains, they agreed the UGA varieties were excellent hosts for their AR novel strains.

Proof of Concept for Novels

'Proof of concept' research was conducted via collaboration by a large UGA collaborative team of agronomists, animal scientists, mycologists, and veterinary scientists working closely with AgR over several years to prove replacing toxic endophytes with novel ones was feasible (Tables 1 and 2). Target traits were 1) strains must be compatible with the host variety and transmissible during seed production; and 2) that toxic ergot alkaloid levels must be nil, yet animal performance and health must be significantly better than E+ and more like E-; but stand survival and yield like traditional E+ tall fescues. These traits are still needed today for any novel fescue variety to be successful.

Table 1. Infection levels and ergot alkaloid concentrations of Jesup and Georgia 5 tall fescue with different novel endophyte strains in initial seed increases when compared to toxic (E+) and endophyte-free (E-) Jesup. Seed then used to

plant survival plots (interplanted into bermudagrass) and plot yield trials over 3 years at different Georgia (USA) locations, Tifton, Eatonton, Athens, and Blairsville (from Bouton *et al*., 2002).

		Initial Seed Fields			**Stand Survival-Plots**		**Dry Matter Yield-Plots**	
Cultivar	**Endophyte**	**Tillers**	**Seed**	**Ergot**	**Tifton**	**Eatonton**	**Athens**	**Blairsville**
	Strain #	*% Endophyte Infection*		*ppb*	*% Stand*		*kg ha^{-1}*	
Jesup	E+	100	96	1,751	36	48	8,635	12,860
Jesup	E-	0	0	0	2	11	7,813	13,525
Jesup	AR502	96	94	39	11	15	8,223	13,547
Jesup	AR510	99	95	18	24	25	8,587	-------
Jesup	**AR542**	**95**	**93**	**8**	**22**	**39**	**9,072**	**12,418**
Jesup	AR572	44	43	91	---	---	------	-------
Jesup	AR577	60	59	170	---	---	------	-------
Georgia 5	AR502	58	56	81	---	---	------	-------
Georgia 5	AR510	76	79	0	---	---	------	-------
Georgia 5	AR542	97	91	0	17	25	8,544	11,769
Georgia 5	AR572	64	55	44	---	---	------	-------
Georgia 5	AR577	72	70	58	---	---	------	-------
				LSD (p<0.05)	16	10	947	1,262
				CV %	79	48	15	11

Table 2. Summary of 3 studies testing Jesup tall fescue with different endophytes status, novel (AR542), endophyte-free (E−), and toxic (E+), during spring grazing on different animal performance and safety parameters as recording for 3 animal systems during spring and autumn grazing at either Eatonton (sheep and beef stocker steers) or Calhoun (beef cow-calf), Georgia (USA); 3-yr means.

Spring Grazing Period											
									Beef Cow-Calf Pairs§		
	Sheep (Lambs)†				**Beef Stocker Steers‡**				**Cow**	**Steer Calves**	**Heifer Calves**
Cultivar	**Ergot¶**	**PRL#**	**Temp**	**Gain**	**Ergot¶**	**PRL#**	**Temp**	**Gain**	**Gain**	**Wean Wt.**	**Wean Wt.**
	ppb	*ng/mL*	*^oC*	*g d^{-1}*	*ppb*	*ng/mL*	*^oC*	*kg d^{-1}*	*kg d^{-1}*	*kg*	*kg*
Jesup E+	1,184^{b*}	7^{b}	40.0^{b}	74^{b}	822^{b}	3^{b}	40.0^{b}	0.31c	0.12^{b}	227^{b}	217^{b}
Jesup E-	31^{a}	123^{a}	39.8^{ab}	122^{a}	0^{a}	94^{a}	39.6^{a}	0.97a	------	------	------
Jesup AR542	29^{a}	146^{a}	39.7^{a}	131^{a}	0^{a}	120^{a}	39.6^{a}	0.78b	0.29^{a}	256^{a}	237^{a}
Autumn Grazing Period											
Jesup E+	2,997^{b}	4^{b}	39.9^{a}	75^{c}	1,208^{b}	1^{b}	40.2^{b}	0.56^{b}	-----	-----	-----
Jesup E-	70^{a}	91^{a}	39.7^{a}	131^{a}	23^{a}	16^{a}	39.9^{a}	0.87^{a}	-----	-----	-----
Jesup AR542	56^{a}	80^{a}	39.9^{a}	106^{b}	28^{a}	11^{a}	39.9^{a}	0.81^{a}	-----	-----	-----

†From Parrish et al. 2003a; ‡From Parrish et al. 2003b; §From Watson et al. 2004; ¶ Ergot alkaloid concentration in the forage; #PRL=Blood prolactin concentration at termination
*Different letters within columns show significant differences (P<0.05).

Of the 5 novel AR strains inoculated into Jesup and Georgia 5 tall fescue all produced ergot alkaloids in the forage dramatically lower than E+ and no different than E- checks, but only AR502, AR510, and AR542 showed high tiller infection levels in the seed increase blocks and especially an ability to transmit endophytes into the harvested seed (Table 1). There were also differences between cultivars with Jesup being more compatible with all 3 strains while Georgia 5 only with AR542. The seed from AR502, AR510, and AR542 in Jesup and AR542 in Georgia 5 were then planted in “accelerated aging” (interplanted into bermudagrass, *Cynodon dactylon* L.), stand survival trials and grazed for 2 full growing seasons at two test locations. Dry matter yield plot trials were also established, and all entries compared against Jesup E+ and E- over 3 years at different test locations. Jesup AR542 consistently gave stand survival and yield similar to E+ but better E- checks (Table 1).

Jesup AR542 was advanced into animal trials and compared to Jesup E+ and E- checks. Three indicators were used to measure animal health, safety, and production: blood prolactin levels (PRL), body temperature, and weight gain. Across different forage-livestock production systems and grazing periods, Jesup AR542 showed responses similar to E- and better than E+ (Table 2). With horses, Jesup AR542 likewise demonstrated results similar to E- with no foaling issues while mares grazing the toxic check experienced agalactia, retained placenta, and even abortion (Ryan et al. 2020). By meeting the two main proof of concept targets, Jesup AR542 was commercialized as Jesup MaxQ® (Pennington Seed Inc.).

Several Novel Varieties Are Now Available

New novels were developed by others using similar protocols. The following novel endophyte tall fescue varieties are now commercially available (listed alphabetically): BarOptima® PLUS E34®, Estancia with ArkShield®, Jesup MaxQ®, Jesup MaxQ II®, Lacefield MaxQ II®, Martin2Protek®, Texoma MaxQ II®, and Tower Protek® (https://content.ces.ncsu.edu/comparison-of-commercially-available-novel-endophyte-tall-fescue-forage-varieties). Patenting, along with quality assurance programs for endophyte viability, make the seed expensive.

Issues and The Alliance for Grassland Improvement

Novel fescue varieties have been available for 20 years yet seed sales are estimated to be a small fraction of the total seed produced for the tall fescue "forage" market. Research programs conclusively demonstrated the problems of fescue toxicosis, and the benefits of converting toxic pastures to novels during that time. Do we need to examine other causes for low adoption besides science-based information? Is there something around human cognitive psychology and biases preventing widespread replacement of current toxic pastures with novels or is it simply seed cost?

One answer to these questions was the formation in 2012 of the Alliance for Grassland Renewal (https://grasslandrenewal.org/) by stakeholders from university, industry, and government. To achieve its mission, the Alliance 1) organizes educational workshops for farmers to understand both the science and practical management of novel tall fescue varieties, 2) establishes rigorous quality testing for certification of commercial products, and 3) promotes incentive programs to assist farmers in the costly but beneficial transition from toxic to novel tall fescue. In this IGC session, a decade of Alliance experience and findings is presented as a model for reducing and eliminating grass endophyte toxicity problems. The model may also hold promise to enhance the adoption of other advanced forage technologies.

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