Complexities of a Breeding Pipeline: Determining the Value of Endophytes in Summer Dormant Tall Fescue

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Introduction

Tall fescue (*Festuca arundinaceum*) is a valuable and broadly adapted forage grass occupying approximately 14 million hectares across the eastern United States. The success of tall fescue is attributed to the presence of *Epicholë coenophiala*, a dominate symbiont that colonizes the above ground plant tissue. There are four interspecific hybrid *Epicholë* taxa that associate with tall fescue, *E. coenophiala*, *Epicholë* sp. FaTG-2, *Epicholë* sp. FaTG-3, or *Epicholë* sp. FaTG-4. Each can be distinguished based on genetic variation that equates to differences found at the alkaloid loci for production of ergot alkaloids, indolediterpenes, lolines, and peramine. These alkaloids provide protection to the plant host from herbivory, but some have also earned a bad reputation, such as the ergot alkaloids that are toxic to grazing livestock. However, a number of elite cultivars have been developed with improved persistence and the inclusion of a *Epicholë* species that is considered safe for grazing livestock. Currently, we are trying to advance the production of tall fescue into Oklahoma and Texas where hot and dry summers can deplete or eliminate stands of tall fescue. Mediterranean (summer dormant, SD) tall fescue has a survival strategy by going dormant during summer thus offering multi-year persistence in stressed environments, unlike the widely planted summer active (continental, SA) tall fescue (Trammell et al., 2018). We have developed a number of clonal-pair populations that differ by presence or absence of endophyte strain to determine if endophyte-infected Mediterranean tall fescue will provide the same benefits with an endophyte that are seen with continental tall fescue. Seeds have been produced from nine populations of clone pairs each E+ and E- line, and we have established sward plots to evaluate persistence of endophyte-infected vs endophyte-free material under intensive grazing and mechanical harvesting. However, breeding with endophytes presents an extra layer of complexity to preserving the integrity of both the symbiont and host throughout the breeding pipeline. We routinely track endophyte infection rate and strain identify, testing both seed and tillers through the greenhouse to the field, using PCR markers developed to distinguish strains based on genetic variation of alkaloid biosynthesis. Currently, research is under way to evaluate summer dormant, clonal pair, tall fescue populations in hot, low rainfall regions to determine the impact of the associated endophyte as a value-added trait in future cultivar development.

Objective

Our objective is to determine the relative merits of ecotype x endophyte combinations across multiple locations where hot and dry summers can deplete or eliminate stands of tall fescue.

Materials and Methods

- To evaluate the importance of endophyte in tall fescue, clone pairs was generated from ten host lines (2x SA and 8x SD) representing 15 unique genotypes/line (n=150, total plants =300).
- Developed isogenic populations using clone pairs by intermating the E+ and E- clones for each genetic background.
- Each clone pair is initiated from a single tiller to reduce the possibility of representing more than one plant genotype.
- Seed was produced in 2015 and 2016 for each isogenic population.
- Isogenic seed tested for the presence or absence of endophyte as well as endophyte type, friendly or common toxic (CTE), using PCR (Fig. 1) specific protocol (Young et al., 2014).
- Trials were established in seven locations across three states in the USA.
- In 2015 and 2016 as grazing trials;
    - Noble's Unit 3 farm in Ardmore, OK (34.10' N, 97.5' W), Windthorst fine sandy loam.
    - Vashti, TX (33.55' N, 98.04' W), Anocon loam.
- In 2017 as clipping plots;
    - Gene Autry, OK (34.17' N, 96.58' W), Dale silt loam.
    - Vashti, TX
    - Fresno, CA (36.45' N, 119.46' W), Hanford sandy loam.
    - Davis, CA (38.52' N, 121.77' W), Mixture of Reiff very fine sandy loam and Yolo silt loam.
    - El Centro, CA (32.48' N, 115.34' W), Holtville silty clay loam.
- In May 2018 as clipping plots:
    - Tulelake, CA (41.96N, 121.47W), Tulebasin mucky silt.
- Experimental design for grazing trials was a randomized block of 1.5 m x 3.1 m sward plots..
- Experimental design for clipping trials was a split with plots consisting of two sown rows each 3.0 m long and spaced 0.2 m apart.
- Trials were sown using a Hege small plot cone-drill into clean seed beds at all locations with a seeding rate of 22 kg ha-1.
- A Trimble GPS unit was used to map each plot in the grazing trials for later accuracy (Fig. 2a, b) when scoring stands after intensive grazing (Fig. 3a, b & c).
- Four treatments were imposed on each clipping trial: continued full irrigation throughout summer or drought conditions combined within each irrigation treatment with either frequent or infrequent harvesting. Thus, within locations, treatments ranged from low stress (infrequent harvest and full irrigation) to high stress (no supplemental water and frequent harvest).
- Data from forage yield trials were taken by cutting plots with a sickle bar plot harvester at a height of approx. 7 cm.
- Sub-samples were collected from each plot during the time of harvest.
- Samples were dried in forced air oven at 60ºC to determine dry weight.
- All plots were adjusted to dry weight basis and include sample weights.

Materials and Methods (continued)

Figure 1. Endophyte characterization pipeline from detection in the plant or seed to confirmation of alkaloid potential.

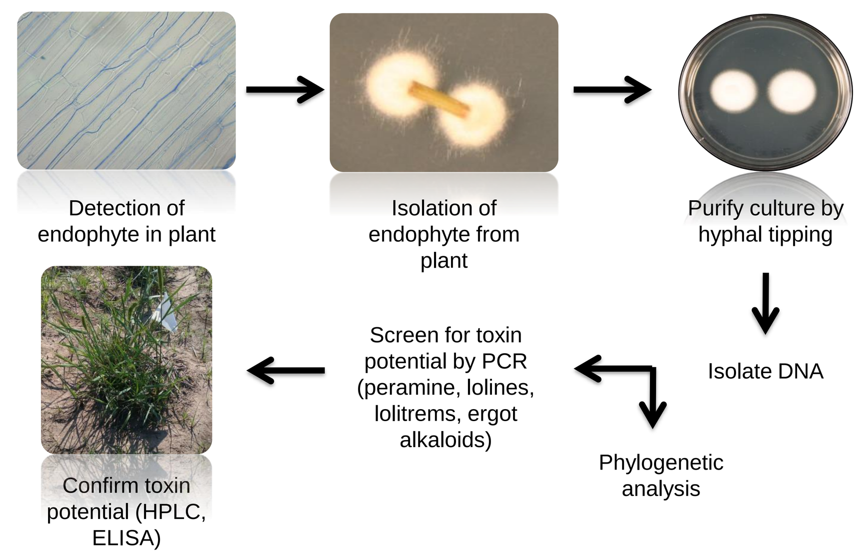


Figure 2. (A & B) Each plot in the field was mapped using a GPS for accuracy during data collection.

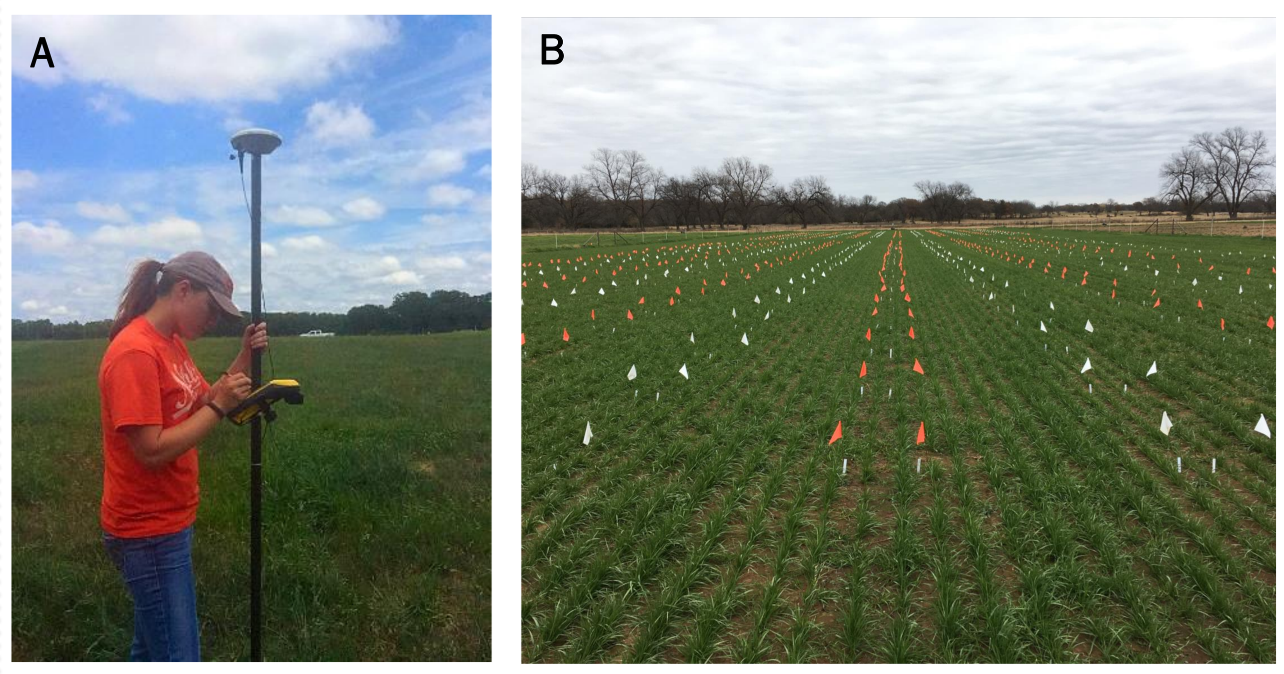
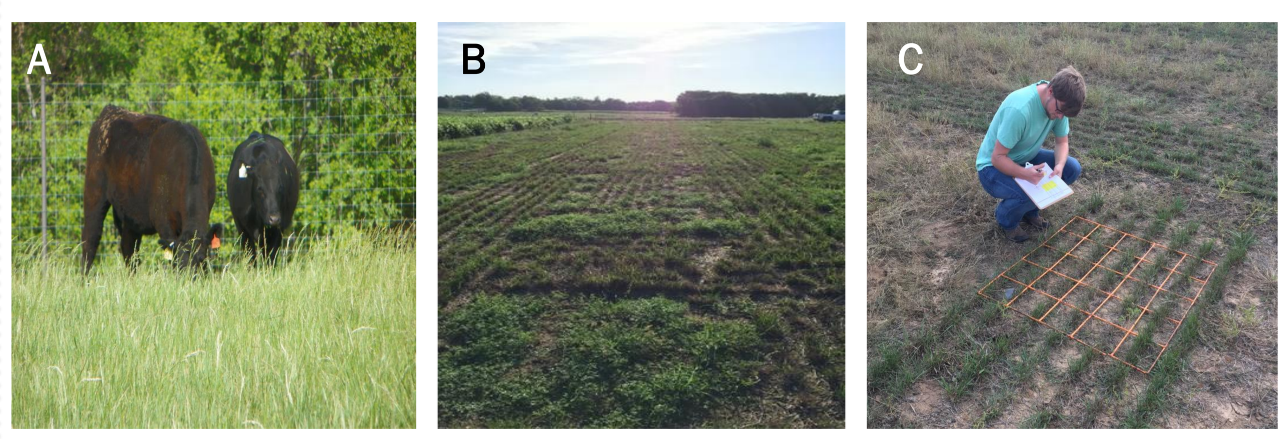


Figure 3. (A) Cattle grazing sward plots established with E+ and E- cloned pair seed. **(B)** Sward plots established with E+ and E- cloned pairs after grazing. **(C)** Stand counts were taken using a grid method as described by Hopkins (2005).



Results

Table 1. Percent stands of cloned pair sward plots under intensive grazing at Noble's Unit 3 farm in Ardmore, OK and at Vashti, TX. Sward plots were established in the fall of 2016.

	Location	Vashti, TX	Vashti, TX	Ardmore, OK	Ardmore, OK
	Trial Type	Grazing	Grazing	Grazing	Grazing
Clonal Isolation	**Endophyte Status**	**Average % Stand** 30-May-17	**Average % Stand** 21-Jun-18	**Average % Stand** 3-Apr-17	**Average % Stand** 13-Jun-18
NFTF 1730	Infected	86	41	100	42
	Nil	70	44	52	29
	50/50	68	36	100	35
Texoma MaxQ II	Infected	48	49	98	95
	Nil	72	22	96	85
	50/50	61	33	90	92
PDF	Infected	54	56	100	92
	Nil	69	56	90	87
	50/50	80	66	96	93
NFTF 1750	Infected	80	54	78	55
	Nil	78	44	100	74
	50/50	67	45	70	44
AGRFA 188 AR605	Infected	25	2	26	22
	Nil	31	2	28	28
	50/50	45	2	48	38
NFTF 1810	Infected	26	11	48	42
	Nil	91	17	50	52
	50/50	58	16	70	65
AGRFA 188 AR589	Infected	26	7	90	55
	Nil	33	11	52	50
	50/50	27	17	58	49
NFTF 1700 AR542	Infected	60	26	100	62
	Nil	68	26	100	31
	50/50	60	22	64	56
NFTF 1700 nil	FT*	88	54	66	33
	Nil	95	64	100	41
	50/50	76	32	38	31

*FT, fungicide treated with the fungicide Proline at 300 g active/ha per application

Results (continued)

- Stands of SD tall fescue were lower than expected during the establishment year (2016) due to poor cold tolerance compared to SA types. Stand data from 2016-17 after intensive grazing are presented in (Table 1).

Table 2. Dry matter yields of isogenic SD and SA populations x endophyte combinations under four stress treatments averaged across two locations, Gene Autry, OK and Vashti, TX in the southern Great Plains, USA. Plots were established in the fall of 2017 and all stands were uniformly excellent. Table represents preliminary data collection from 2018 only.

			Water treatment							
			Drought				Irrigated			
			Harvest treatment							
			Infrequent		Frequent		Infrequent		Frequent	
Cultivar/germplasm	Ecotype	Endophyte	E+	E-	E+	E-	E+	E-	E+	E-
			Mg ha^{-1}							
NFTF 1700	SD	AR502	4.3	4.9	4.2	4.4	5.1	6.4	5.9	6.0
NFTF 1700	SD	AR542	4.1	5.0	4.1	4.5	4.9	6.9	6.0	6.3
NFTF 1730	SD	AR584	4.4	5.2	4.2	4.7	5.9	6.9	5.8	6.3
NFTF 1810	SD	AR584	4.0	4.8	4.5	4.6	5.7	6.9	5.8	6.2
Texoma	SA	AR584	6.7	6.1	6.9	6.0	6.9	5.6	7.1	6.3
PDF	SA	CTE	6.0	6.0	6.0	5.7	9.0	5.6	8.6	6.1
Mean			5.0	5.3	5.0	5.1	6.3	6.4	6.5	6.2
LSD (0.05)			**0.3**	**0.3**	**0.4**	**0.4**	**0.2**	**0.4**	**0.3**	**ns**

Table 3. Dry matter yields of isogenic SD and SA populations x endophyte combinations at three locations in California evaluated across 3 harvests during the summer of 2018 under full irrigation and infrequent harvest treatment.

Cultivar/germplasm	Ecotype	Endophyte	Davis	Fresno	El Centro
			Mg ha^{-1}		
NF1700	SD	E-	6.5	5.8	**7.1**
NF1700	SD	AR542	7.0	8.5	**7.2**
Texoma	SA	E-	7.7	6.7	**8.4**
Texoma	SA	AR584	10.02	9.0	**8.8**
NF1810	SD	E-	7.7	7.4	**7.3**
NF1810	SD	AR584	9.5	6.9	**7.3**
PDF	SA	E-	9.3	8.3	**8.3**
PDF	SA	CTE	9.4	9.0	**8.9**
NF1730	SD	E-	6.1	7.4	**8.8**
NF1730	SD	AR584	8.3	7.2	**9.4**
LSD (0.05)			**0.8**	**0.8**	**0.6**
Contrasts					
E+ vs. E-			S	S	NS
E-			7.5	7.1	8.0
E+			8.8	8.1	8.3
SD vs SA			S	S	S
SD			7.5	7.2	7.9
SA			9.1	8.2	8.6
SD nil vs SD endo			S	NS	NS
SD nil			6.7	6.9	7.8
SD endo			8.3	7.5	8.0
SA nil vs SA endo			S	S	NS
SA nil			8.5	7.5	8.3
SA endo			9.7	9.0	8.9

S, Significant; NS , non-significant

- From initial harvest data collected during Fall of 2018 at Gene Autry, OK and Vashti, TX locations, yields under irrigation were higher on average for both SD and SA germplasms than under drought stress regardless of the presence or absence of fungal endophyte (Table 2).
- Yields of SD E+ germplasm were lower than that of the E- SD germplasm across all treatments (Table 2).
- Yields of SA E+ germplasms were higher than that of SA E- germplasms regardless of treatment (Table 2).
- This study and data collection will continue over the next several years at each site for multiple location analysis (Table 2).
- On average, at all sites in California, E+ germplasms yielded more than E- germplasms (Table 3).
- Stands of SA germplasms were declining across all sites in California when compared to SD germplasms regardless of endophyte infection (data not shown).

Conclusions

We should caution against over interpretation of the clipping data since this study is in its infancy and data collection will continue over the next several years at each site for multi-location analysis. The results of these trials will give us clear indications of the value of several different endophytes to yield and persistence of tall fescue under a wide diversity of environmental stresses. Information gathered from these trials will aid in recommending germplasm throughout both regions and help focus future breeding efforts.

References

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