Evaluation of stress response characteristics facilitated by endophytes in commercially available perennial ryegrass, tall fescue and fine fescue cultivars

Hannah Rivedal[1], Pear Intasin[2], Navneet Kaur[2], Ruying Wang[2] and Alec Kowalewski[2]

[1]United States Department of Agriculture Forage Seed and Cereal Research Unit
[2]Oregon State University

OTRF Report – December 2022

Perennial Ryegrass: Spring 2022, plants were sampled from the 2016 Perennial Ryegrass NTEP trial. We sampled 51 cultivars – the 6 standard cultivars and 45 cultivars that have been made commercially available – from the trial. For each cultivar, 5 random leaf samples per plot of 5 leaves each were collected from each replicate (5 samples from 3 reps, 15 samples per cultivar). Collected samples were maintained at -80°C until processing. All 51 cultivars have been extracted for total nucleic acids with the method described by Dellaporta et al. (1983). Multiplex polymerase chain reaction (PCR) developed by Vikuk et al. 2019 will be used to determine endophyte alkaloid profile starting in January.

Fine Fescue: Fall 2022, we sampled seeds and plants from the Fine Fescue A-LIST trial. In both cases, samples from all 25 cultivars were sampled. Seeds (125 individual seeds per cultivar) that were saved at planting, were placed in a 4°C refrigerator until processing. For processing, three replicates of 25 seeds each were extracted using the same nucleic acid extraction method as above (Dellaporta et al. 1983). The Vikuk et al. (2019) primers were used on the seed extracts. Specifically, these primers are used to detect the presence of *Epichloë* endophytes, and if present, their alkaloid patterns that are associated with toxicity to pests. Results indicate that 48% of cultivars in the A-LIST trial have an endophyte infection (Table 1) and all positive samples had detectable ergot alkaloid gene regions. Samples with detectable peramines and indole diterpenes were less prevalent, and no samples had detectable loline gene regions. These alkaloid profiles will be monitored in plant samples over the life of the A-LIST trial. The first sample of plant tissue was also conducted (5 samples from 3 reps, 15 samples per cultivar as for perennial ryegrass), plant samples have yet to be processed for PCR but will be early next year.

Citations

Dellaporta, S., L. Wood, and J. Hicks. 1983. A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter.* 1: 19–21.

Vikuk, V, Young, CA, Lee, ST, Nagabhyru, P, Krischke, M, Mueller, MJ, Krauss, J. 2019. Infection rates and alkaloid patterns of different grass species with systemic *Epichloë* endophytes. *Appl Environ Microbiol* 85:e00465-19. https://doi.org/10.1128/AEM.00465-19

Table 1. Multiplex PCR results for endophyte presence and alkaloid profile of seeds of each cultivar in the A-LIST fine fescue trial.

Sample	Cultivar	Fine Fescue Type	Seed Endophyte Status[A]	Endophyte Alkaloid Presence[B]			
				Peramine	Loline	Ergot Alkaloid	Indole Diterpene
1	Blue Hornet	Sheep	-[C]	-	-	-	-
2	Jetty	Hard	+	+	-	+	+
3	Cardinal II	Strong Creeping	-	-	-	-	-
4	Compass II	Chewings	+	+	-	+	+
5	PPG-FRC 130	Chewings	+	+	-	+	-
6	PPG-FRC 127	Chewings	+	+	-	+	+
7	PPG-FFR 127	Strong Creeping	+	+	-	+	-
8	PPG-FL 128	Hard	-	-	-	-	-
9	PPG-FFR 134	Strong Creeping	+	-	-	+	+
10	PPG-FFR 132	Strong Creeping	-	-	-	-	-
11	Conductor	Chewings	+	+	-	+	+
12	Clarinet	Hard	+	+	-	+	+
13	Chorus	Strong Creeping	+	+	-	+	-
14	Woodall	Chewings	+	+	-	+	+
15	Marvel	Strong Creeping	-	-	-	-	-
16	Minimus	Hard	-	-	-	-	-
17	DLFPS-FRR-3128	Strong Creeping	-	-	-	-	-
18	DLFPS-FL-3104	Hard	-	-	-	-	-
19	Ruddy	Strong Creeping	-	-	-	-	-
20	Quatro	Sheep	-	-	-	-	-
21	Chantilly	Strong Creeping	-	-	-	-	-
22	SR 5130	Chewings	-	-	-	-	-
23	Leonidas	Hard	-	-	-	-	-
24	Leeward	Chewings	+	+	-	+	+
25	DLFPS-FRC-3105	Chewings	+	+	-	+	+
Average Incidence (%)[D]			48	44	0	48	36

[A] Presence (+) or absence (-) of the TEF1a housekeeping gene, indicating an endophyte infection of seed; [B] Presence or absence of one of the four major alkaloid producing genes in *Epichloë* endophytes that are toxic to pests; [C] Presence (+) or absence (-) determined after three replications of PCR (25 seeds in each extraction); [D] Average incidence expressed as a percentage of positive cultivars out of the total number of cultivars for each gene region evaluated in the multiplex PCR reaction.