

Article

Endophytic Fungal Infection of Meadow Fescue in the Driftless Area of the Upper Mississippi River Valley: Impacts on Agronomic Fitness

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Abstract: Meadow fescue, *Schedonorus pratensis* (Huds.) P. Beauv., has recently been discovered as a common but previously unknown pasture grass in the Driftless Area of the upper Mississippi River Valley, USA. Preliminary data also indicated that many meadow fescue pastures were infected with an endophytic fungus, *Epichloë uncinata* (W. Gams, Petrini & D. Schmidt) Leuchtm. & Schardl. Therefore, the objective of this study was to determine if the endophyte impacts agronomic fitness of the host meadow fescue. Meadow fescue plants from eight farm sites were intensively sampled, and endophyte infection levels were determined to range from 82 to 95%. Paired endophyte-infected (E+) and endophyte-free (E−) meadow fescue subpopulations from each collection site were then created, and were subsequently compared for greenhouse and field drought tolerance, forage mass, and persistence under frequent defoliation. There was no impact of the endophyte under a wide range of drought conditions for either greenhouse or field studies. Furthermore, there was a small forage-mass-enhancement effect in the E+ subpopulation for only one of the eight collection sites. The only consistent effect was an average of 9% increased ground cover (persistence) in endophyte-infected meadow fescue under frequent defoliation. As per other studies, enhanced root growth, fungal-disease resistance, and/or reduced insect feeding could be mechanisms for this increased survivorship. We conclude that the meadow fescue endophytes present in the Driftless Area do not help protect their host from drought or provide any consistent forage-growth enhancement; however, we found evidence that the endophyte provides some protection against frequent defoliation at low residual sward heights.

Keywords: *Schedonorus pratensis*; *Festuca pratensis*; *Epichloë uncinata*; breeding; drought; grazing; defoliation; stress



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1. Introduction

Meadow fescue (*Festuca pratensis* Huds.; *Schedonorus pratensis* (Huds.) P. Beauv.) is a grass commonly grown in Nordic and alpine pastures for its combination of winter hardiness, nutritive value, and high dry-matter intake [1–5]. Outside of these regions, meadow fescue abundance has decreased in recent years [6]. Researchers reported that Eurasian meadow fescue populations had reduced species-level genetic diversity compared to related taxa such as perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Schedonorus arundinaceus* (Schreb.), and interpreted that genetic pressure was the result of range contraction [7,8]. In response, some have recommended conservation of permanent grasslands as reservoirs of functional genetic diversity [9].

The Driftless Area is an unglaciated portion of the upper Mississippi River Valley that consists of approximately 6.2 M ha in Wisconsin, Minnesota, Iowa, and Illinois, USA [10,11]. Following the Pleistocene Glaciation, this region consisted primarily of a mixture of oak savanna, prairie, and sparse deciduous forest [12–15]. The first European settlers were

largely miners from Wales, Switzerland, Italy, and Scandinavia who required a nascent agricultural industry to support their communities [16–18]. This was largely pastoral agriculture with cattle and sheep that were supported by pastures established with seeds collected and transported from their home countries. Following World War Two, agricultural industrialization and mechanization within the USA led to dramatic changes to agrarian practices within the Driftless Area [19,20]. Because it was unglaciated during the Pleistocene Period, the Driftless Area consists of deep valleys, thousands of kilometers of spring-fed streams, and steep hillsides. Pastures were plowed, and thousands of hectares of land were converted to grain production. Pastures that survived the plow were largely oak savanna remnants [15] that were generally inaccessible for logging, typically located on hilltops, along steep gullies, near stream beds, and adjacent to other wetlands.

In 2002, a heretofore unknown forage grass was discovered that was dominant in a few of these remnant pastures near Mineral Point, WI [21]. Using molecular markers, researchers identified this unknown grass as meadow fescue [22]. Surveys conducted during the next few years documented meadow fescue in remnant pastures on approximately 400 distinct farms within the Driftless Area (Figure 1). Surveys and interviews with landowners documented that these pastures were not established with modern seeded cultivars anytime during recent memory within the past 70–80 years, and it was tentatively concluded that these meadow fescue populations were brought to the Driftless Area by European settlers. Circumstantial evidence for this conclusion was that the highest concentration of documented meadow fescue pastures were located within a few kilometers of U.S. Highway 151, which roughly follows the historic Military Ridge Road, the main thoroughfare for settlers to this region prior to 1900. Those researchers further determined that there were at least four distinct introductions of meadow fescue to this region, three originating from known European populations and one from a previously unknown European population [21].

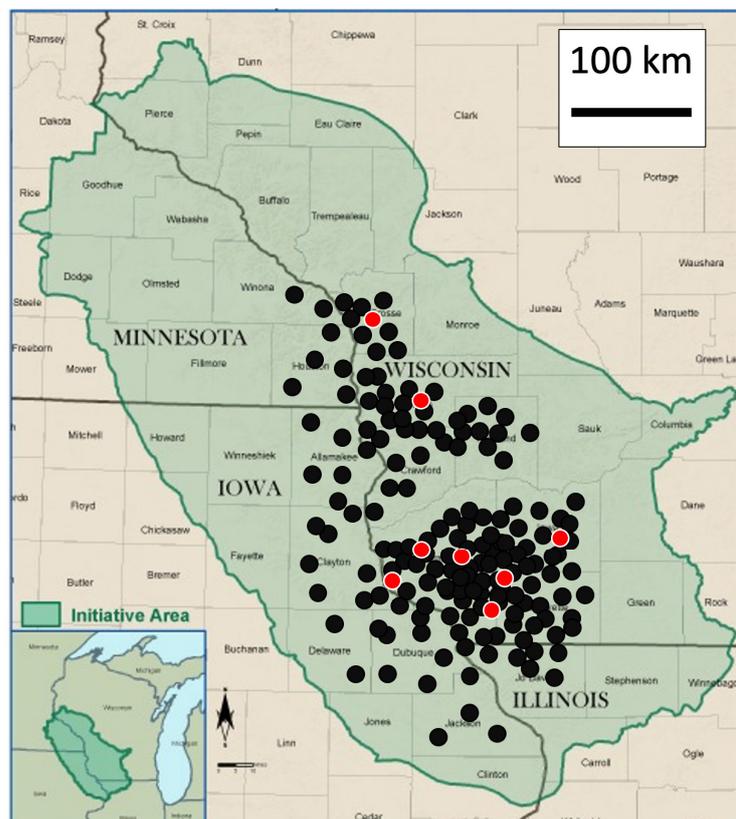


Figure 1. Map of the Driftless Area showing the locations of approximately 400 distinct farms with meadow fescue pastures. The eight sites that were the subject of the experiments described in this paper are shown as red circles.

Subsequent sampling of meadow fescue pastures within this region identified plants that were infected with an endophytic fungus, *Epichloë uncinata* (W. Gams, Petrini & D. Schmidt) Leuchtm. & Scharl. It is now well documented that many grasses have developed symbiotic associations with fungi including fungi that systemically infect grass shoots. These grass endophytes, defined as fungi that live their entire life cycle within the aerial portion of the host grass, form nonpathogenic, systemic, and usually intercellular associations [23]. Endophyte-infected grasses express a range of adaptations to biotic (reviewed in [24]) and abiotic stresses, including drought (reviewed in [25,26]). As a result, endophyte-infected grasses are more competitive than noninfected grasses and often thrive better than noninfected grasses [23]. Furthermore, endophyte-infected grasses have been reported to have increased root mass, tiller weight, herbage mass [27–29], and seed production [30]. Therefore, it is possible that meadow fescue's persistence in the Driftless Area is in part due to these E+ enhanced fitness adaptations. Thus, identification of these endophyte-infected pastures led to our hypothesis that the fungal endophyte had a positive impact on fitness of meadow fescue in the Driftless Area, conferring greater vigor, drought tolerance, and/or tolerance to frequent defoliation. Therefore, the research objectives of this study were (1) to investigate the frequency of *Epichloë uncinata* infection rates on meadow fescue in the Driftless Area and (2) to quantify the impact of the endophyte on agronomic fitness (e.g., morphology, drought and defoliation tolerance, and forage mass) of the meadow fescue host.

2. Materials and Methods

2.1. Endophyte Frequency and Germplasm Development

Eight farms were chosen as the collection sites for these experiments; they were chosen at random to represent a broad geographic sample of the Driftless Area and a subset of the 71 sites studied by Duncan et al. [21] (Figure 1 and Table 1). Approximately 1000 plants were randomly sampled from each of the eight farms in 2011. Taken from healthy meadow fescue pastures that were largely monocultures, these plants were transplanted to eight isolated blocks near Arlington, WI in 2011. The presence of *Epichloë uncinata* were determined in two tiller sections from at least 200 random plants per farm, 6 weeks after transplanting, using the monoclonal antibody immunoblot assay “Phytoscreen Field Tiller Endophyte Detection Kit” (Cat. #ENDO797-3; Agrinostics Ltd., Co., Watkinsville, GA, USA) according to the manufacturer's description (Table 1). This assay was described by Hiatt et al. [31], and has been reported to be accurate for detecting the presence of *Epichloë uncinata* in meadow fescue [32,33]. The endophyte infection rates shown in Table 1 were based on all plants for which the two independent tiller sections agreed (n = 205–263).

Table 1. Location, habitat, and endophyte infection rates (based on monoclonal antibody immunoassay on shoot tissues) for the eight collection sites (i.e., farms) included in the analysis of endophyte-free (E−) vs. endophyte-infected (E+) meadow fescue.

Site/ Farm	Location	Latitude	Longitude	Habitat	Number of Plants	Endophyte Infection Rate
						%
1	Coon Valley	43°41.60' N	91°0.25' W	Full sun, north facing slope	257	89.6
2	Cassville	42°45.47' N	91°1.40' W	Deep shade, steep hillside	243	85.2
3	Hidden Valley	42°47.65' N	90°15.65' W	Full sun, south facing slope	205	94.8
4	Fennimore	42°57.33' N	90°37.56' W	Full sun, rolling hills	231	90.4
5	Platte River	42°44.17' N	90°38.62' W	Full sun, riverine	248	84.0
6	Linden	42°53.05' N	90°14.77' W	Deep shade, oak savanna, hilltop	263	91.9
7	Bear Creek	43°34.25' N	90°36.53' W	Full sun, riverine	211	87.9
8	Little Grant River	42°53.32' N	90°49.54' W	Full sun, riverine	217	82.0

The eight blocks of approximately 1000 plants were allowed to pollinate in isolation from each other to generate eight seed populations in summer 2012. The eight seed populations were created by bulking equal masses of seeds of each half-sib family within a block. Seed bulks of the eight populations were then split at random into two subsets, one of which was stored in a freezer at $-4\text{ }^{\circ}\text{C}$ and the other on a laboratory bench at $22\text{ }^{\circ}\text{C}$ for 2 years, the former to promote survival of *Epichloë uncinata* and the latter to encourage mortality of the endophyte. Following these differential storage conditions, approximately 500 seedlings from each of the 16 subpopulations were germinated from the stored seeds, and seedlings were tested for presence-absence of *Epichloë uncinata* using the immunoblot assay described above. This endophyte evaluation verified that the 2012 seed lots stored under freezing conditions retained their E+ status of 82 to 95% infection, while those stored at room temperature in the laboratory had endophyte infection levels of 0 to 7% (e.g., E-). Immediately following these determinations, all 16 of the subpopulation seed lots were vacuum packed and stored at $-4\text{ }^{\circ}\text{C}$ for use in the subsequent experiments.

2.2. Morphological and Seed-Production Evaluation

One hundred and fifty confirmed E+ and E- plants originating from each of the eight populations were selected and transplanted into eight isolated checkerboard crossing blocks, with one for each farm source, near Arlington, WI in May 2015. The checkerboard design consisted of alternating E+ and E- plants, so that every E+ plant had four E- neighbors and vice versa with a plant spacing of 0.9 m. Neighboring blocks from different farms were 20 m apart. In May 2016, all crossing blocks were fertilized with 55 kg N ha^{-1} of ammonium nitrate fertilizer. The following traits were measured for all plants ($n = 300$) in each of the eight nurseries in both 2016 and 2017: heading date as the day for which about half of the panicles had fully emerged from the boot; plant height to the highest point after all plants were fully headed; and plant circumference at the base of the plant. In addition, plants were allowed to be openly pollinated and seed hand harvested in 2016 and 2017 for each plant. Seed was dried in a forced-air dryer for 7 days at $30\text{ }^{\circ}\text{C}$, then threshed, cleaned, and its mass determined on an individual-plant basis.

2.3. Drought-Tolerance Evaluation

2.3.1. Drought in the Greenhouse

Random seeds of the 16 subpopulations were germinated and used to establish greenhouse-grown seedlings. All seedlings were grown in cylindrical plastic cones measuring $2.5 \times 15\text{ cm}$, and using a commercial potting mix as the soil substrate. When seedlings were approximately 80–100 days old, they were randomly assigned to drought treatments. There were four drought treatments: 0, 7, 14, and 21 days in drought. Drought treatments were assigned to whole plots in a split-plot arrangement of a randomized complete block design with six replicates. Sub-plots consisted of the 16 subpopulations and each experimental unit consisted of a single row of six seedlings. Drought treatments were all initiated on the same day by withholding water/irrigation for the specified period of time. Forty-two days after initiation of drought treatments, biomass was harvested from each experimental unit and the number of surviving plants was recorded. Samples were dried at $60\text{ }^{\circ}\text{C}$ for 7 days, weighed, and their dry biomass recorded. Biomass was expressed on both an experimental unit basis and a per-plant basis. This entire experiment was repeated three more times in succession over the course of a 20-month time period in 2017–2019, with each repetition requiring approximately 5 months.

2.3.2. Deficit-Irrigation Evaluation

The 16 meadow fescue subpopulations were planted in a line-source sprinkler experiment [34] to evaluate the interactions between deficit irrigation and endophyte infection status. The experimental site and layout for this line-source sprinkler were described by Waldron et al. [35]. In brief, the experiment was located at the Utah State University (USU) Evans Experimental Farm, approximately 2 km south of Logan, UT, USA ($41^{\circ}45'\text{ N}$,

111°8' W, 1350 m above sea level). This site is found within the semiarid Central Great Basin region of the western USA and is characterized by hot, dry summers with most of the annual precipitation as winter-time snowfall that is stored in reservoirs and used for irrigated crop production [36]. Experimental plots were arranged as a modified split-plot design with four replications, two on each side of a line-source irrigation line, and five water levels applied as non-randomized strips (i.e., as a whole plot) [34,35]. Plots were planted on 21 August 2018 with a five-row Wintersteiger cone seeder (Wintersteiger Corporation, Salt Lake City, UT, USA) at a rate of 148 pure live seeds per linear meter of the row (approximately 13.7 kg ha⁻¹). The plots were oriented perpendicular on both sides of a line-source irrigation system, and in five successively more distal 2.0 m long ranges (e.g., corresponding to the 5 water levels) separated by 1 m mowed alleys (see Figure 2).

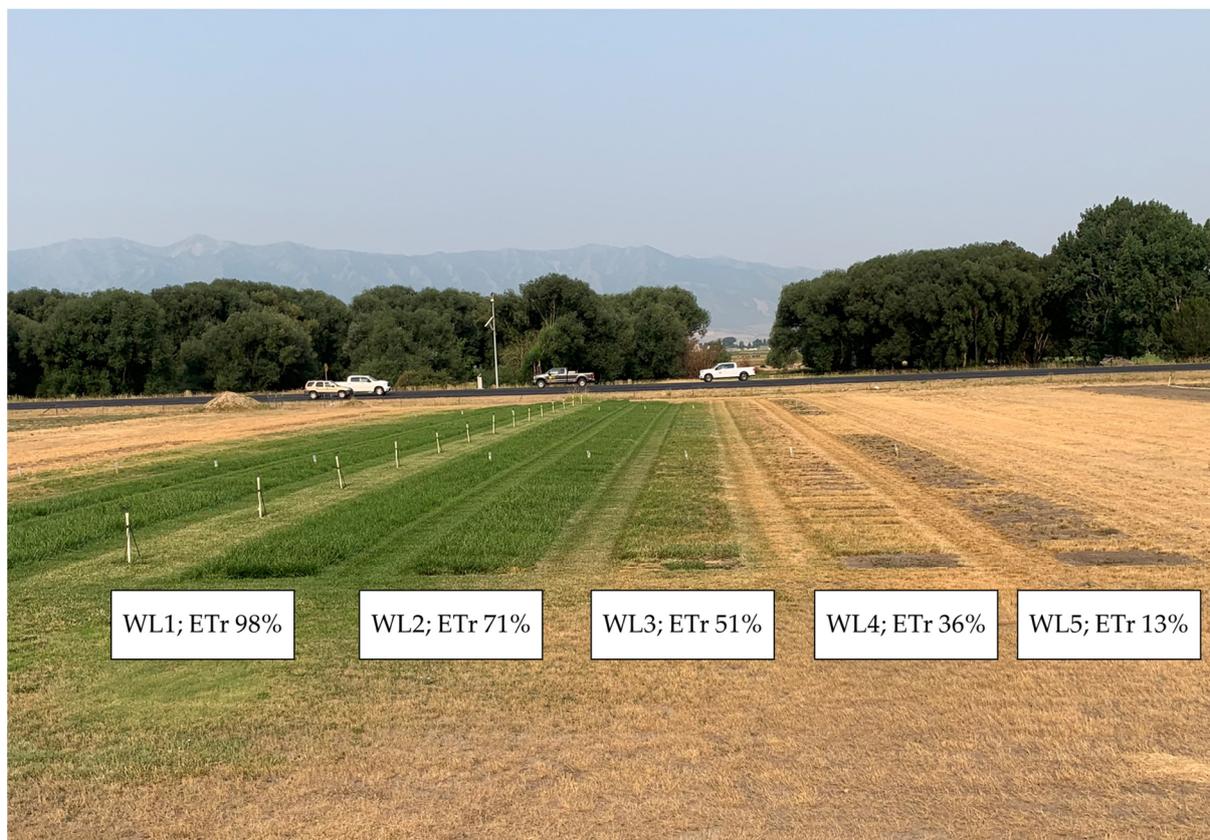


Figure 2. Meadow fescue line-source deficit-irrigation experiment conducted 2019–2021 near Logan, UT. Plots were oriented perpendicular on both sides of a line-source irrigation system in five successively more distal 2.0 m long ranges (i.e., water levels as %ET replacement; ETr) separated by 1 m mowed alleys. At this late-summer date, the most distal plots are completely dormant (36% ETr) or dead (13% ETr). Photograph taken on 21 July 2021 at USU Evans Farm by author BLW.

Plots were irrigated uniformly as needed during the establishment (August–September 2018, May–July 2019). Plots were mowed regularly during 2019, and irrigation treatments were initiated in August 2019 to establish a soil water gradient. Irrigation water plus rainfall were monitored from May through to September in 2020, and May through to August in 2021. Reference evapotranspiration (ET_o) values for these time periods were obtained from the Utah Climate Center, using the Hargreaves equation, at a weather monitoring station approximately 3.2 km from the Evans research farm (USC00425194), and irrigation was applied weekly to reach 100% ET replacement at the water level nearest to the sprinkler. Fertilizer applications of 56 kg N ha⁻¹ of ammonium nitrate were made prior to the first harvest and after the second and third harvests in 2020 and after the first two harvests in 2021. During 2020 and 2021, plots were harvested to a 10 cm stubble height with an RCI flail

forage harvester (RCI Engineering, Mayville, WI, USA). All plots were harvested on the same day on 4 June, 4 August, and 24 September 2020, and 8 June, 13 July, and 16 August 2021. The first harvest was conducted when growth in the 100% ET replacement water level was between the vegetative and elongation stage and subsequent harvests occurred when plant regrowth reached 30 to 35 cm in height. Forage samples from each plot were dried at 60 °C in a forced-air oven and used to estimate dry forage mass.

2.4. Forage-Mass and Persistence Evaluation

2.4.1. Field Evaluation: Wisconsin

Field experiments were established for the 16 subpopulations at Arlington, WI in April 2018. The seeding rate was 500 pure live seeds m^{-2} , which was approximately equal to 11 kg ha^{-1} . Plots were arranged in two independent experiments, each containing all 16 subpopulations with 8 replicates in a randomized complete block design and a split-plot randomization, in which farm (e.g., collection) sites were whole plots and endophyte status consisted of the paired subplots. In 2019 and 2020, one experiment managed to collect data on forage yield of a three-harvest management system. Plots were fertilized with 55 kg N ha^{-1} of ammonium nitrate fertilizer prior to each of the six growth periods. Each entire plot was harvested in early June, early August, and late October using a flail-type harvester and samples dried at 60 °C in a forced-air oven to constant weight, and were used to estimate dry-matter and dry forage mass.

The adjacent experiment at Arlington was managed to create a defoliation stress, as suggested by the research of Brink et al. [2]. All plots were mowed to a residual sward height of 5 cm whenever the canopy reached an average height of 8 to 10 cm, resulting in 12 to 15 mow events per year. To add to the stress, nitrogen fertilizer was added at the same rate and timings as the adjacent forage-yield experiment, generally on a frequency of every-other mow event, encouraging rapid regrowth and more frequent mowing treatments. The only variable measured was ground cover in May 2019, 2020, and 2021, using a 50-point grid in which each cell measured 15 × 15 cm [37].

2.4.2. Field Evaluation: Utah

The 16 meadow fescue subpopulations were also included in an evaluation of the U.S. National Plant Germplasm System (NPGS) meadow fescue collection at two Utah locations. The two locations were the USU Evans Experimental farm (described above) and USU Lewiston Intermountain Pasture Research Farm (41°57'01.85" N, 111°52'15.75" W, elev. 1369 m, 46 cm annual precipitation and 56.1 precipitation d/year) located near Lewiston, Cache County, UT, USA. The Lewiston site has a similar semiarid climate as the USU Evans Farm site, but a sandier soil classified as Kidman Fine Sandy Loam and Lewiston Fine Sandy Loam (both are Coarse-loamy, mixed, superactive, mesic Calcic Haploxerolls). The evaluations were established in the spring of 2018 by transplanting greenhouse-grown seedlings to the field in 7-plant (Evans) or 5-plant (Lewiston) plots with 0.5 m between plants and 1 m between rows. Field design was a randomized complete block design (RCB) with three (Evans) or two (Lewiston) replicates. Plots were sprinkler irrigated uniformly as needed during the establishment year. In 2019 and 2020, irrigation was limited to one application of 2 to 3 h each week resulting in an average growing season ET replacement of 33 to 40%. Fertilizer applications of 56 kg N ha^{-1} of ammonium nitrate were made prior to the first harvest and immediately after the second and third harvests. During 2019 and 2020, plots were harvested to a 10 cm stubble height with an RCI flail forage harvester (RCI Engineering, Mayville, WI, USA). Plots were harvested on 10 June, 31 July, and 3 October 2019, and 10 June and 5 August 2021 at Evans farm and one day later at Lewiston. Random forage samples used to estimate percentage of dry matter were dried at 60 °C in a forced-air oven to constant weight and used to compute the forage mass of each plot.

2.5. Statistical Data Analysis

All experiments were analyzed with linear mixed models [38]. The distribution of residual values were all sufficiently close enough to normality that no data transformations were required. Collection/Farm sites, endophyte levels, and their interaction were fixed effects in all analyses. Single degree-of-freedom contrasts were used to test the effect of endophyte (E+ vs. E−) independently for each of the eight farm sites.

For the parental evaluation in the checkerboard design, year was a random effect modeled with a homogeneous compound symmetry covariance structure. For the greenhouse drought experiment, the four runs or repetitions of the experiment were treated as a random effect. For the two field experiments at Arlington, Wisconsin, year was treated as a random effect modeled with a homogeneous compound symmetry covariance structure. For the field experiment at Evans and Lewiston, Utah, year, location, and all of their interactions were treated as fixed effects because of the dryland nature of these locations and the expectation that there would be significant declines in yield and survivorship from one year to the next. For the line-source irrigation experiment at Logan, Utah, year was treated as a random effect modeled with a homogeneous compound symmetry covariance structure. Blocks and all interactions with years were random effects; irrigation treatments and all interactions with farm sites and endophyte levels were fixed effects.

3. Results

3.1. Endophyte Frequency, and Morphological and Seed Production Evaluation

Pasture sampling determined that the majority of meadow fescue plants were infected with the *Epichloë uncinata* endophyte from all eight farms. Overall endophyte infection frequency averaged 88% and ranged from 82 to 95% (Table 1). These results confirmed preliminary findings that meadow fescue and *Epichloë uncinata* associations were widely distributed in the Driftless Area. Evaluation of the subsequent 16 subpopulations found that E+ plants had a later flowering date than E− plants from seven of eight collection sites, whereas E+ plants from three collection sites were shorter ($p = 0.01$), and from five sites had larger crowns ($p = 0.01$) than their corresponding E− plants (Table 2). In contrast, E+ plants from all eight collection sites had on average 26% greater ($p = 0.10$ – 0.01) seed yield than their E− counterparts (Table 2).

Table 2. Flowering, morphological, and seed production trait means of parental plants sampled from *Epichloë uncinata* endophyte-free (E−) or endophyte-infected (E+) subpopulations of meadow fescue.

Site/Farm	Heading Date		Plant Height		Plant Circumference			Seed Yield				
	E−	E+	E−	E+	E−	E+		E−	E+			
	Day of Year		----- cm -----		----- cm -----			----- g plant ^{−1} ----				
1	144	145	**	80	71	**	59	60	68	78	*	
2	143	144	**	78	88	**	61	61	89	155	**	
3	143	144	**	86	83		55	60	**	55	83	**
4	143	144	**	78	79		65	70	**	57	65	†
5	145	145		84	79	**	64	66		55	82	**
6	146	147	**	89	84	**	61	67	**	58	67	†
7	145	146	**	85	84		61	68	**	76	87	*
8	146	147	**	84	83		65	69	**	69	42	**

†, *, ** E−, and E+ subpopulations were significantly different at $p < 0.10$, 0.05 , or 0.01 .

3.2. Drought-Tolerance Evaluation

Despite a wide range of drought conditions for both greenhouse and line-source irrigation experiments, there were no significant effects associated with collection sites, *Epichloë* endophyte infection, or their interaction on biomass production. On average across collection sites and endophyte status, Wisconsin greenhouse drought of 14 days without

irrigation resulted in 70% less plant survival and 78% less plant biomass than control plants (Table 3), whereas 21 days without irrigation resulted in near complete death and zero biomass production (Table 3). Likewise, the Utah line-source irrigation evaluation resulted in 44, 62, and 78% less average biomass at 51, 36, and 13% ET replacement, respectively, compared to 98% ET replacement, with plant death eventually occurring at 13% ET replacement (Table 3 and Figure 2).

Table 3. Mean values of 16 meadow fescue subpopulations for traits measured under drought-stress conditions in Wisconsin greenhouse environments and Utah field-based (line-source irrigation, LS) environments.

Greenhouse Drought Treatment	Plant Survival	Total Dry Biomass	Dry Biomass per Plant	Utah LS Deficit Irrigation	Forage Mass
	%	g	g	% of ET ¹	Mg ha ⁻¹
None (control)	99.0 ± 0.20	3.03 ± 0.05	0.51 ± 0.01	98	10.99
7 days without water	89.4 ± 1.09	1.62 ± 0.03	0.31 ± 0.01	71	14.89
14 days without water	28.9 ± 1.44	0.24 ± 0.01	0.11 ± 0.01	51	6.16
21 days without water	0.4 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	36	4.18
				13	2.43

¹ Deficit irrigation applied as percent of evapotranspiration (ET) replaced by weekly irrigation events.

3.3. Field Forage Mass and Persistence

Forage mass was significantly higher ($p = 0.01$) for the E+ compared to the E- subpopulation only for collection site #2, but this effect was consistent in both Wisconsin and Utah evaluations (Table 4). And though the evaluation conditions of the Wisconsin and Utah evaluations were very different, the E+ subpopulation of collection site #2 had a substantially greater mass than its E- counterpart (i.e., 28 and 116% more, respectively) (Table 4). On average, E- subpopulations had 9.4% less ($p = 0.01$) ground cover than E+ subpopulations after three years of frequent mowing (Table 4 and Figure 3). Furthermore, this endophyte infection effect ($p = 0.01$) on stand persistence was consistent across all eight collection sites (Table 4).

Table 4. Mean values of *Epichloë uncinata* endophyte-free (E-) or endophyte-infected (E+) subpopulations of meadow fescue evaluated in field experiments conducted in Wisconsin or Utah.

Site/Farm	Forage Yield: Wisconsin ¹			Forage Yield: Utah ²			Ground Cover: Wisconsin ³			
	E-	E+	Diff.	E-	E+	Diff.	E-	E+	Diff.	
	Mg ha ⁻¹		%	--- g plant ⁻¹ ---		%	--- % ---		%	
1	7.64	7.95	4.1	204	270	32	73.5	81.0	10	**
2	7.09	9.06	27.8	224	484	116	87.2	94.5	8	**
3	7.06	7.24	2.5	180	190	6	74.8	81.5	9	**
4	7.17	7.19	0.3	214	196	-8	89.2	94.8	6	**
5	6.75	6.83	1.2	184	192	4	83.5	92.0	10	**
6	7.44	7.55	1.5	156	178	14	77.2	88.0	14	**
7	6.89	6.80	-1.3	220	196	-11	75.5	81.5	8	**
8	7.48	7.19	-3.9	270	224	-17	59.8	65.8	10	**

** E- and E+ subpopulations were significantly different at $p < 0.01$. ¹ Total yield over three harvests which is averaged over 2 years. ² Spaced-plant evaluation irrigated at 33% ET replacement; total yield over two or three harvests and averaged over 2 years and two locations. ³ Ground cover after three growing seasons of frequent mowing.



Figure 3. Paired plots of meadow fescue subpopulations from Farm Site #1 with *Epichloë uncinata* endophyte-infected subpopulation on the right; and endophyte-free subpopulation on the left, showing typical differences in ground cover between endophyte infection status. Distance between painted lines is 0.9 m. Photograph taken on 26 October 2020 at Arlington, WI, by author MDC.

4. Discussion

Epichloë uncinata endophyte infection of the eight intensively sampled pastures ranged from 82 to 95% (Table 1). These observed infection rates are slightly higher than the average infection rates of 69 to 91% observed for approximately 200 wild meadow fescue accessions collected from a wide geographic range in northern Europe, where this species is native [39,40]. *Epichloë* infection rates of meadow fescue cultivars are known to be highly variable, ranging from 0 to 95% [41], suggesting that the high infection rates observed in natural meadows and these Driftless Area pastures may be functional, with the fungal endophyte helping to protect the host from some unknown stress.

Endophyte-infected meadow fescue plants tended to be slightly later in flowering, slightly shorter, and had slightly larger crowns than endophyte-free plants (Table 2). These effects were not consistent for all eight collection/farm sites, suggesting that they may not be fitness related per se, but may be related to the physiological cost of meadow fescue plants hosting the endophyte. It has been reported that the endophyte may carry a physiological cost that can reduce plant vigor, in particular reducing shoot mass, number of tillers, or number of reproductive panicles [42]. These authors also demonstrated that these effects on morphology and vigor were not consistent across different host cultivars and environments [42]. However, despite the reduction in plant height and crown size for some farm sites, we found that seed yield tended to be enhanced by *Epichloë* endophyte infection, although this effect was highly variable across the eight farm sites (Table 2). Likewise, the impact of *Epichloë uncinata* endophyte on the seed yield of meadow fescue was highly variable in a prior study, with a very strong genotype \times environment interaction [42]. Yet, meadow fescue seed production was also enhanced by *Epichloë* endophyte infection in another study, but only after treatment with an insecticide that eliminated natural attacks

by root aphids [43]. Unlike these observations for meadow fescue, the positive impact of *Epichloë* endophytes on the seed production of tall fescue is greater and more consistent than for meadow fescue [30]. Rather, the results for meadow fescue are similar to those for perennial ryegrass, for which *Epichloë* endophytes had highly inconsistent and variable impacts on seed production [44,45].

Both drought experiments, the Wisconsin greenhouse and the Utah line-source irrigation system, resulted in a wide range of responses for meadow fescue, including nearly complete mortality in the greenhouse and a 78% reduction in forage mass in the field (Table 3). Despite the successful application of a wide range of drought conditions for both experiments, there were no significant effects associated with collection sites, *Epichloë uncinata* endophyte infections status, or their interaction. Endophyte infection of meadow fescue has been shown to increase root dry matter in a greenhouse study, a trait that could be a mechanism of drought avoidance [46]. However, the effect size was similar under well-watered and drought stress conditions, so it was likely a physiological enhancement of root growth, but not necessarily a mechanism of enhanced drought avoidance or tolerance. *Epichloë* fungal endophytes positively impact several mechanisms of both drought avoidance and tolerance of host plants from many different perennial grass genera, including *Festuca*, *Lolium*, and *Schedonorus* [47–49]. Examples include more extensive root systems, enhanced control of transpiration, enhanced water storage in host tissues, accumulation of assimilates, and enhanced osmotic adjustments during drought [23]. While tall fescue and perennial ryegrass have been extensively studied and documented, there are no other reports of investigations related to the impact of *Epichloë* infection on drought avoidance or tolerance of meadow fescue. Based on our results, the safest conclusion is that there is no effect.

Forage mass was higher for the E+ compared to the E– subpopulation only for one of the collection sites (i.e., farm site #2), but this effect was consistent across all locations in both Wisconsin and Utah (Table 4). While there is a large body of evidence indicating that endophyte-infected genotypes of tall fescue and perennial ryegrass have superior forage yield or growth rates compared to endophyte free genotypes [44,50–53], this is the first indication that this phenomenon may exist in meadow fescue. The fact that it was observed for only one of the eight collection sites (e.g., farms) is consistent with results from tall fescue and perennial ryegrass that show highly inconsistent effects that are highly dependent on host genotype and perhaps on endophyte genotype [44,51].

Meadow fescue populations from all eight collection sites had significant stand losses under the frequent defoliation regime (Table 4). As a reference, ground cover under the three-harvest forage-mass evaluation in the adjacent field experiment was 100% for all plots of all subpopulations. Stand losses over three years of intensive and frequent defoliation in this experiment were greater than those observed by Brink et al. [2] for either a three-harvest or six-harvest defoliation system. The frequent defoliation system of Brink et al. [2] was meant to mimic, as closely as possible, the management-intensive grazing system generally recommended in the North Central USA: five to six grazing events at a sward height of 20 to 25 cm, leaving approximately 10 cm as a residual sward height. In that study, lowering residual sward height from 10 to 5 cm reduced ground cover from 84 to 71% for meadow fescue [2]. In the current study, ground cover under frequent defoliation was the only trait we measured in these experiments with a consistent response to *Epichloë* endophyte infection status across all eight collection/farm sites (Table 4). This result suggests that the endophyte may provide the host with some protection under defoliation systems that are more intensive than typically recommended, i.e., more frequent defoliation and lower residual sward heights. In the survey and exploration of the Driftless Area described by Duncan et al. [21], meadow fescue was frequently found in pastures that fit this description, sometimes appearing to be more-or-less continuously grazed.

While it seems clear that the *Epichloë uncinata* endophytes present in the Driftless Area do not help protect their host meadow fescue from drought or provide any consistent growth enhancement (except for the one exception from farm site #2), we found evidence to

conclude that the endophyte provides some level of protection against frequent defoliation at extremely low residual sward heights. The mechanism for these responses is not obvious, suggesting several avenues for further research. Endophytes of both tall fescue and perennial ryegrass are well known to protect their hosts against a range of both fungal pathogens and insect predators [24,54]. The meadow fescue endophyte also provides a significant level of protection to its host against stand loss due to snow mold [55] and feeding by fall armyworm [56], mealybugs, and aphids [57], any of which could aid in survivorship under frequent and low defoliation stresses. In addition, enhanced root growth, root mass, and competitive ability associated with the endophyte of meadow fescue [46] could be additional mechanisms for enhanced survivorship under the stresses induced by frequent and low defoliation.

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