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Fusarium Species Associated with Tall Fescue Seed Production in Oregon

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Abstract

Seed samples were collected from 15 commercial tall fescue seed production fields and examined for *Fusarium* spp. The percentage of seeds from which *Fusarium* spp. were recovered ranged from 0 to 32%, while disinfesting seeds with 3% hydrogen peroxide reduced the recovery of *Fusarium* to 7% or less. The predominant *Fusarium* spp. isolated from the tall fescue seeds included *F. avenaceum*, *F. culmorum*, *F. pseudograminearum*, and *F. sambucinum*. Greenhouse inoculations of tall fescue panicles with *F. avenaceum*, *F. culmorum*, and *F. pseudograminearum* resulted in higher seedborne rates of each respective *Fusarium* sp. than that recovered from noninoculated plants. Seeds recovered from panicles treated with *F. avenaceum* or *F. pseudograminearum* had significantly lower germination rates relative to panicles sprayed with water or a suspension of *F. culmorum*. Our work confirms that *Fusarium* spp. decrease seed germination and expands the pathogen list to include *F. avenaceum* and *F. pseudograminearum*.

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) seed production is an important industry in Oregon. In 2002, 65,862 ha of tall fescue in Oregon yielded 114 million kg of seed, valued at $86 million (11). Nearly all the hectares are planted as certified seed, requiring a high level of purity and germination. In 1998, the Oregon State University (OSU) Seed Laboratory noted reduced germination, below the 90% minimum germination requirement for seed certification, in tall fescue seed samples submitted for germination determination as required for seed certification. *Fusarium* sporodochia were observed on some seeds, and an association of *Fusarium* with reduced germination in tall fescue was suspected (S. C. Alderman, *unpublished data*). *Fusarium heterosporum* (4,9) and *F. culmorum* (5) have been previously reported on tall fescue seeds and roots. The impact of *Fusarium* spp. on viability of tall fescue seeds has not been established, nor has it been shown whether infection by a *Fusarium* sp. during flowering will lead to increased seedborne frequency of that species.

Germination of grass seed is typically evaluated under 15/25°C night/day temperatures but in some cases an elevated temperature regime of 20/30°C night/day is used to shorten the duration of the germination test (2). It is unknown if either temperature regime favors *Fusarium* seed or seedling infection, but the 20/30°C regime was discontinued at the OSU Seed Laboratory due to observations of greater fungal infections of seeds during germination under the warmer temperature regime.

The objectives of this research were to: (i) recover and identify species of *Fusarium* from commercial tall fescue seed produced in Oregon; (ii) determine if *Fusarium* infections can be controlled by surface disinfestation; (iii) determine if germination is decreased under an elevated incubation temperature; and (iv) evaluate the potential of *Fusarium* spp. as seed pathogens when introduced during flowering of tall fescue.

Evaluation of Tall Fescue Seeds Produced in Commercial Fields

During 1998, 15 25-g samples of tall fescue seed samples with reduced germination rates were obtained from the OSU Seed Laboratory. Each seed sample represented a separate field. From each sample, 50 nontreated seeds were picked at random and embedded into solidified Nash medium (7) supplemented with aureomycin (6). Plates were incubated at 24°C with only ambient light for 21 days. An additional set of 50 seeds from each sample was disinfested with 3% hydrogen peroxide for 60 min, triple-rinsed with sterile, distilled water, and cultured as above. Putative *Fusarium* colonies were transferred from the Nash medium to potato dextrose agar (PDA) and carnation leaf agar (CLA) (8). Cultures were incubated under fluorescent lamps supplemented with black light in a 12-h photoperiod at 24°C (8). Each putative *Fusarium* colony was identified to species according to the system of Nelson et al. (8), with the exception of *F. pseudograminearum* which was formerly known as *F. graminearum* Group I and lacks homothallic perithecial production (1). The experiment was repeated. Means were calculated and a paired *t*-test was used to compare disinfested to nontreated seed (SAS 8.02, Cary, NC). A subset of isolates was purified by the single-spore method and stored on silica gel at 5° C (10).

Since the recovery of *Fusarium* spp. was similar between experimental replicates, data from experimental replicates were combined. The percentage of seed from which *Fusarium* spp. were recovered ranged from 0 to 32% (Table 1). Disinfestation with hydrogen peroxide reduced recovery of *Fusarium* from 0 to 32% to 0 to 7%. The predominant *Fusarium* sp. recovered from nontreated seeds was *F. avenaceum* (Table 2). *Fusarium sambucinum*, *F. pseudograminearum*, and *F. culmorum* also were commonly recovered from nontreated seeds; *F. oxysporum*, *F. equiseti*, *F. semitectum*, *F. lateritium*, and *F. proliferatum* were rarely isolated. *Fusarium avenaceum* and *F. sambucinum* were the predominant species recovered from disinfested seeds (Table 2). Since seed disinfestation significantly reduced the frequency of seedborne *Fusarium* spp. recovered, the majority of *Fusarium* spp. was likely present on seeds as surface contaminates or as superficial infections.

Table 1. Germination percentage of tall fescue seeds and percentage that yielded *Fusarium* spp.

Seed sample no.[a]	Cultivar	Germination[b]	Percent nontreated seeds that yielded *Fusarium* spp.[c]	Percent H_2O_2-treated[d] seeds that yielded *Fusarium* spp.
163887	RG-93 (Bravo)	81	32	4 ***
165016	Leprechaun	86	32	5 ***
163222	El Dorado	89	30	3 ***
163827	RG-93 (Bravo)	82	29	2 ***
164654	Bonzai	82	29	3 ***
162769	Titan	91	28	3 ***
163891	RG-93 (Bravo)	78	22	7 ***
162888	Titan	89	11	1 ***
162687	Orygun	87	8	0 **
163204	LRF-983	84	6	1 *
163226	Rebel III	86	5	1
162937	Avalon	82	3	0
163227	Rebel III	88	1	1
162276	Coyote	79	0	0
162855	Houndog	89	0	0

[a] OSU Seed Certification Laboratory record number.

[b] Determined by the OSU Seed Certification Laboratory.

[c] Based on two replicates of 50 seeds per sample in each experiment.

[d] Seeds were disinfested in 3% hydrogen peroxide for 60 minutes. Means of H_2O_2-treated seeds that are significantly different from nontreated seeds are labeled, according to paired *t*-test, with: *** for $P = 0.005$; ** for $P = 0.01$; and * for $P = 0.05$.

Table 2. Frequency of *Fusarium* spp. recovered from nontreated and disinfested tall fescue seeds.[a,b]

Seed sample no.[c]	Seed treatment	*F. avenaceum*	*F. culmorum*	*F. sambucinum*	*F. semitectum*	*F. pseudograminearum*	*F. equiseti*	*F. oxysporum*	*F. proliferatum*	*F. lateritium*	Total frequency
163887	nontreated	17	2	11	1	0	1	0	0	0	32
	disinfested	2	0	1	0	1	0	0	0	0	4
165016	nontreated	15	2	10	1	3	0	1	0	0	32
	disinfested	4	0	1	0	0	0	0	0	0	5
163222	nontreated	12	4	10	0	3	1	0	0	0	30
	disinfested	1	0	2	0	0	0	0	0	0	3
163827	nontreated	15	0	13	0	0	0	1	0	0	29
	disinfested	0	0	2	0	0	0	0	0	0	2
164654	nontreated	13	4	7	0	2	0	1	0	2	29
	disinfested	2	0	0	1	0	0	0	0	0	3
162769	nontreated	6	7	10	0	4	0	1	0	0	28
	disinfested	2	0	0	0	0	0	1	0	0	3
163891	nontreated	8	4	9	0	1	0	0	0	0	22
	disinfested	2	1	3	1	0	0	0	0	0	7
162888	nontreated	4	3	2	0	0	0	2	0	0	11
	disinfested	1	0	0	0	0	0	0	0	0	1
162687	nontreated	2	1	5	0	0	0	0	0	0	8
	disinfested	0	0	0	0	0	0	0	0	0	0
163204	nontreated	2	1	0	0	0	1	1	1	0	6
	disinfested	0	0	0	0	0	0	0	1	0	1
163226	nontreated	2	1	2	0	0	0	0	0	0	5
	disinfested	0	0	1	0	0	0	0	0	0	1
162937	nontreated	2	0	1	0	0	0	0	0	0	3
	disinfested	0	0	0	0	0	0	0	0	0	0
163227	nontreated	0	0	1	0	0	0	0	0	0	1
	disinfested	1	0	0	0	0	0	0	0	0	1
162276	nontreated	0	0	0	0	0	0	0	0	0	0
	disinfested	0	0	0	0	0	0	0	0	0	0
162855	nontreated	0	0	0	0	0	0	0	0	0	0
	disinfested	0	0	0	0	0	0	0	0	0	0

[a] Based on two replicates of 50 seeds per sample in each experiment.

[b] Frequency of recovery of *Fusarium* spp. as % of seeds in each sample.

[c] OSU Seed Certification Laboratory record number.

To evaluate the effect of elevated temperature during germination, nontreated seeds from each sample were placed on moist germination paper in germination plates (15-cm diameter, 2.5-cm height), cold-treated at 5°C for 5 days, and then incubated at either 15/25°C or 20/30°C night /day under a 16-h photoperiod. Germination counts were made on days 7 and 14. A seed was considered germinated when the radicle was equal to or greater than twice the length of the caryopsis. One hundred seeds were evaluated from each sample in each of two experimental replicates. Mean percentage germination was calculated and a paired *t*-test was used to compare germination between the temperature regimes on day 14 for each seed sample.

Since seed germination was not significantly different between experimental replicates, data from experimental replicates were combined. Seed germination on day 7 was generally lower under the 20/30°C than the 15/25°C temperature regime, but by day 14, germination was similar in both temperature regimes (Fig. 1). Since the level of recovery of *Fusarium* spp. from certified seed samples was not correlated with germination percentage, other microorganisms or environmental or host factors not categorized in this study may have contributed to the reduced germination.

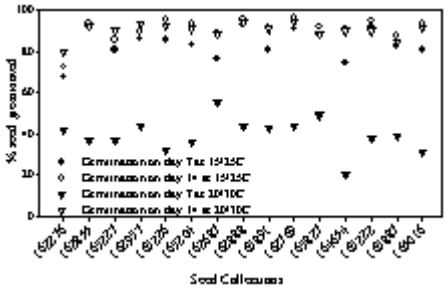

Fig. 1. Tall fescue seed germination under two temperature regimes; 15/25°C and 20/30°C. Based on 2 replicates of 100 seeds per sample at each temperature regime in each experiment.

Greenhouse Plant Inoculations

Tall fescue seeds (cultivar Fawn) were sown in 10-cm diameter pots containing a pasteurized greenhouse soil mix (1 part sandy loam:1 part peat:1 part pumice, pH 6.6). Pots were placed in a greenhouse with 21/18°C day/night with a 14-h photoperiod (provided by high-pressure sodium lamps). After seedling emergence, plants were thinned to one per pot. At the 8-leaf stage, plants were vernalized in a growth chamber for 5 weeks at 5°C with an 8-h daylength, and then plants were returned to the greenhouse. Fertility was maintained with Osmocote slow release fertilizer.

Four isolates of *F. pseudograminearum*, three of *F. avenaceum*, and one of *F. culmorum*, all obtained from tall fescue seed and stored on silica gel crystals, were revived on CLA under the light and temperature regimes previously described. Inoculum was increased by transferring 5-mm agar plugs from 10- to 14-day-old CLA cultures to 50 ml of sterile Difco potato broth in 150-ml flasks. Flasks were placed on a rotary shaker at 100 rpm and incubated for 4 days with no additional lighting. Cultures were minced with a hand-held homogenizer, filtered through four layers of cheesecloth, and diluted with sterile distilled water to a concentration of 10^6 conidia per ml using a hemocytometer. The four isolates of *F. pseudograminearum* and the three isolates of *F. avenaceum* were bulked together by species. The control was sterile distilled water. At the beginning of flowering, plants were inoculated using a hand-held atomizer and the conidial suspension was sprayed onto each head until near run-off. On each plant, a different panicle was inoculated with each *Fusarium* sp. and one additional panicle was sprayed with distilled water (control). The greenhouse inoculations were conducted three times. A completely randomized design was used, with 16, 55, and 36 plants for the first, second, and third replicate, respectively, for a total of 107 plants. Plant number was not equal in each replicate due to lack of panicles on some plants. Panicles were hand-threshed when seed was mature. Five seeds from each panicle were arbitrarily placed into

amended Nash medium as previously described. After 14 days, putative *Fusarium* colonies were subcultured onto CLA and PDA for species identification.

Twenty-five seeds from each panicle were placed in germination plates, cold-treated at 5°C for 5 days, and then incubated under a 15/25°C day/night regime with a 16-h photoperiod. Germination counts were made on day 14. A seed was considered germinated when the radicle was equal to or greater than twice the length of the caryopsis. *Fusarium* spp. were tested for significant effects (P = 0.05) on percentage seed carrying *Fusarium* spp. using a general linear model (SAS 8.02, Cary, NC) for an analysis of variance of percentage seeds yielding *Fusarium* spp., germination rates, and the percentage of seedlings that died during the germination evaluation; means were compared with Tukey's W statistic.

Panicles inoculated with each *Fusarium* sp. resulted in a corresponding increase (P = 0.05) in numbers of seeds yielding *Fusarium* (Table 3). *Fusarium avenaceum* and *F. pseudograminearum* infestations (31.2 and 27.5% seeds, respectively) were significantly greater (P = 0.05) than that of *F. culmorum* infestation (17.4% seeds). The same *Fusarium* sp. used in panicle inoculation was recovered from seed from the corresponding inoculated panicles (*data not shown*). When panicles were sprayed with distilled water, *F. oxysporum* was detected in less than 5% of the seeds tested.

Table 3. Germination and mortality rates of tall fescue seeds from inoculated panicles

Panicle treatment	% seeds[a] with *Fusarium* spp.	% seeds[b] germinated at day 5	% seeds germinated at day 14	% germling mortality[c]
F. avenaceum	31.2 a	46.7 a	84.3 b	24.9 a
F. culmorum	17.4 b	51.8 a	90.5 a	14.5 b
F. pseudograminearum	27.5 a	41.8 a	76.5 c	30.7 a
Water-control	4.9 c	50.7 a	91.2 a	5.7 c

[a] 535 seeds per treatment (five seeds per tiller from each plant in three replicates for a total of 107 plants) were sampled for *Fusarium* spp. Means labeled with the same letters are not significantly different (P = 0.05) according to Tukey's W statistic.

[b] 2675 seeds per treatment (25 seeds per tiller, 107 plants) were used in the germination evaluation.

[c] The percentage of germlings which died during the 14 days of study.

Germination rates of seeds from inoculated panicles were similar on the fifth day of germination (Table 3). However, on day 14, significantly fewer (P = 0.05) seeds from panicles sprayed with *F. pseudograminearum* had germinated compared to the other panicle treatments. Percentage germination of seeds harvested from the *F. avenaceum* inoculation was also significantly reduced relative to the water-control. No reduction in germination resulted from the *F. culmorum* treatment. Germling death was observed during germination, and the percentage mortality was significantly greater in all *Fusarium* treatments than the water control (Table 3).

Discussion

It has been previously reported that tall fescue seed can be colonized by *F. heterosporum* (4,9) and *F. culmorum* (5), resulting in seed decay or damping-off problems; *F. heterosporum* was not detected in this study. Our work confirms that *Fusarium* spp. introduced during tall fescue flowering can decrease germination of the subsequent seed crop below the 90% minimum germination required for seed certification, and our work expands the pathogen list to include *F. avenaceum* and *F. pseudograminearum*. Additional studies are necessary to determine whether the presence of *F. avenaceum* or other *Fusarium* spp. will cause seedling diseases in the field and whether the stage of seed development at time of inoculation affects subsequent *Fusarium* presence on seed.

Fusarium spp. can colonize plant residues and live as saprophytes. During the 1940s through the 1980s, most grass-seed fields were burned post-harvest to control diseases and remove residues. Legislation passed during the 1990s placed strict limits on the annual acreage that can be burned. Increased levels of grass residues in many of the tall fescue fields may be supporting larger populations of pathogenic *Fusarium* spp., and perhaps a greater prevalence of *Fusarium* spp. on seed. Producers may experience periodic germination problems, detected during seed certification, due to *Fusarium* spp. Seed treatments have been shown to suppress seedborne *Fusarium* spp. (5) and protect germlings against pathogenic soilborne *Fusarium* spp. (3), however these treatments are not applied prior to seed certification.

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