AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT, FUNDING CYCLE 2019 – 2021

TITLE: The Role of Seedborne *Fusarium* in Wheat Root, Crown, and Foot Rot

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EXECUTIVE SUMMARY: Numerous species of the fungus *Fusarium* are known to be soilborne, including *F. culmorum* and *F. pseudograminearum*. Strains of *Fusarium* can survive on wheat stubble and straw as well as in the soil, infecting plants during subsequent seasons. Certain strains of *Fusarium* can infect roots and crowns of wheat, causing disease of spring and winter wheat. Root, crown, and foot rot disease is common in dryland winter wheat and no-till annual spring cereals throughout the Pacific Northwest. This disease is associated with high fall soil temperatures, low fall soil moisture, and moisture stress after flowering; all conditions that are occurring more frequently in wheat production regions across the state of Oregon. Although information is known about aspects of seedborne *Fusarium*, most of the reported information pertains to Fusarium head blight. The role of *Fusarium* on seed is much less clear in terms of the development of root, crown, and foot rot in wheat. We examined six wheat varieties for the presence of *Fusarium* and detected *Fusarium* species in all varieties. Three of the six wheat varieties, Kaseberg, Ovation, and Stephens, had *Fusarium* on 13% or greater of the seed assayed. The predominate *Fusarium* species recovered was *F. proliferatum* and replicated greenhouse pathogenicity tests showed that isolates of this fungus in the soil cause rot of the seminal roots and mesocotyl tissues.

OBJECTIVES: Determine the identity, prevalence, and pathogenicity of *Fusarium* species associated with seed of wheat varieties commonly grown in Oregon.

PROCEDURES: Samples of wheat seed were provided by researchers at OSU (Robert S. Zemetra and Mark Larsen, CSS). One hundred wheat seeds of each of six wheat varieties (Duet, Jasper, Kaseberg, Ovation, Stephens, and Tubbs 06) were embedded in a *Fusarium*-selective agar media in each of two runs. Microfuge tubes containing individual seeds were incubated for up to four weeks, allowing *Fusarium* to grow out from infected seeds at room temperature with no auxiliary lighting. Putative *Fusarium* colonies were transferred from the *Fusarium*-selective medium to microbiological media used for morphological identification, and these subcultures were incubated for classic *Fusarium* identification. Each putative *Fusarium* colony was identified to species by Ocamb according to morphology of spores and spore-bearing structures. Total genomic DNA was isolated from these representative colonies identified microscopically as *Fusarium*, and PCR was used to amplify a barcoding sequence from the genomic template. The sequence of these PCR products was used to confirm the identity of *Fusarium* strains. Identified *Fusarium* species from the first run were put into purified stock cultures for the subsequent greenhouse studies on the pathogenicity of representative *Fusarium* strains.

Six isolates of *Fusarium* collected from wheat seed were transferred to autoclaved millet grain and incubated for four weeks in spawn bags with a filter patch under ambient conditions. Steam-pasteurized planting medium was mixed 1:1 volume ratio with Sunshine Mix #4 and an Oregon native sandy loam. Plastic pots (4” x 4”) were filled with the medium and *Fusarium*-treated pots were amended with colonized millet that contained an individual *Fusarium* isolate. A set of pots

were left non-inoculated and used as the nontreated control. Nontreated wheat 'Duet' seeds were sown by hand with one seed planted per pot and pots were placed on a greenhouse bench under 400-watt metal halide lights. Nine pots per isolate were used in each experimental run of the greenhouse study. Run 1 was planted on 14 Oct 2020 and run 2 was planted on 10 Feb 2021. Approximately eight weeks after sowing, wheat plants were destructively sampled. Shoot weights were recorded, and roots were visually rated for rot of mesocotyl and roots. Tissues with rot symptoms were excised from affected plants, rinsed in 10% bleach for 30 seconds followed by a distilled water rinse, and embedded in petri dishes containing a *Fusarium*-selective medium. *Fusarium* growth was evident on the medium after seven days of incubation on the lab benchtop. Molecular and morphological identification was used for confirmation of the *Fusarium* species obtained from symptomatic plants.

SIGNIFICANT ACCOMPLISHMENTS:

In the evaluation of *Fusarium* species on wheat seed, *Fusarium* was recovered from all six wheat varieties examined and the average incidence from the two runs are shown in Table 1. Three varieties, Kaseberg, Ovation, and Stephens, had a seedborne *Fusarium* incidence that averaged 13% or greater while Duet, Jasper, and Tubbs had below 10% incidence of *Fusarium* on seed. Seven different *Fusarium* species were obtained from wheat seed, but *F. proliferatum* was the predominate species and was the only one recovered from all six wheat varieties examined (Table 2). Stephens had the greater number of different *Fusarium* species recovered as well as the highest seedborne incidence of *Fusarium* overall.

Table 1. Average incidence of *Fusarium* on wheat seed

Variety	% seed with *Fusarium*[z]
Duet	4.5
Jasper	5.5
Kaseberg	13.0
SY Ovation	18.5
Stephens	19.5
Tubbs 06	1.0

[z] Means are based on the results of assaying 100 seeds of each wheat variety in each of two runs.

Table 2. Average incidence of various *Fusarium* spp. on seed of six wheat varieties

	% seed with *Fusarium* spp.[z,y]							
Variety	**Fculm**	**Feq**	**Foxy**	**Fprol**	**Fsamb**	**Fsol**	**Fvert**	**Fus spp.**
Duet	0	0	1	3	0	0	0	0.5
Jasper	0	0	0	4	0	1.5	0	0
Kaseberg	0	0	1	11.5	0.5	0	0	0
Ovation	0	0	0.5	14.5	0	2.5	1	0
Stephens	3	1.5	0.5	8.5	0	4	0.5	1.5
Tubbs 06	0	0	0	1	0	0	0	0

[z] Means are based on the results of assaying 100 seeds of each wheat variety in each of two runs.

[y] The various *Fusarium* species recovered from wheat seed included F. culmorum (Fculm), F. equiseti (Feq), *F. oxysporum* (Foxy), *F. proliferatum* (Fprol), F. *sambucinum* (Fsamb), *F. solani* (Fsol), *F. verticillioides* (Fvert), and *Fusarium* isolates obtained that were not identified to species (Fus spp.).

The wheat seed yielded more than just *Fusarium* in the assays, as Ocamb has found over the past 30 years of research on seeds placed on a *Fusarium*-selective medium; selective media do not prevent growth of all other microbes in most cases. However, wheat seeds samples examined yielded a higher than expected number of non-*Fusarium* fungal species as well as oomycetes, and most of these additional microbes were not identified. Periodically, *Fusarium* isolates were overrun by the other microbes after transfer, and although *Fusarium* was observed, it was impossible to type to the exact species. These strains are denoted as *Fusarium* spp. Some non-*Fusarium* fungi were identified (*Alternaria*, *Penicillium*, and *Rhizoctonia* spp.). (Table 3).

Table 3. Average incidence of various fungi and oomycetes on seed of six wheat varieties

	% seed infected [z]			
Variety	***Alternaria***	***Penicillium***	***Rhizoctonia***	**Oomycetes**
Duet	1.5	1	0	0.5
Jasper	1	0.5	0	0
Kaseberg	0	0	0	0
Ovation	3	0	0.5	1.5
Stephens	0	4	0	0
Tubbs 06	0	0	0	0

[z] Means are based on the results of assaying 100 seeds of each wheat variety in each of two runs.

In the greenhouse studies, six different isolates of *F. proliferatum* were evaluated and the majority of wheat plants grown in *F. proliferatum*-inoculated soil exhibited rot of mesocotyl and seminal roots (Figure 1). Non-inoculated control plants showed limited rot of the mesocotyl and seminal roots (Table 4). Wheat growing in non-inoculated soil in the greenhouse studies likely had disease on the seminal roots and mesocotyl as a result of seedborne pathogens due to the seed not being treated by either fungicides or seed disinfestation techniques. Tissue samples excised from symptomatic mesocotyls and roots exhibited *Fusarium* growth after seven days of incubation on the *Fusarium*-selective medium. Further molecular and morphological identification confirmed that *F. proliferatum* was recovered from infected seminal roots as well as the mesocotyl of plants grown in inoculated pots in the greenhouse studies.

Table 4. Incidence of rot on seminal roots and the mesocotyl of 'Duet' wheat in greenhouse studies

***Fusarium* isolate** [z]	**% plants with rot of seminal roots and mesocotyl** [y]
Non-inoculated	28 b
Fprol 3-09	89 a
Fprol 51-84	89 ab
Fprol 03-20	72 ab
Fprol 03-76	94 a
Fprol 02-35	94 a
Fprol 01-52	89 ab

[z] Strains of *Fusarium proliferatum* obtained from wheat seed that were used to inoculate pots of wheat in the greenhouse.
[y] Means within the column followed by the same letter are not significantly different at $P = 0.05$ by Fisher's F-protected least significant difference (LSD) test. $R^2 = 0.35$.

Fig. 1. Photograph showing decay of the mesocotyl and seminal roots (area outlined in red) of wheat growing in the presence of *Fusarium proliferatum*. Photograph by Taylor A. Bates, 2020.

The experimental run x isolate were significant in terms of plant shoot weights so shoot weight data are presented separately for experimental runs 1 and 2. In the first experimental run, the overall average shoot weight was 6.4g while in the second run it averaged 9.7g. Generally, the presence of *Fusarium* appeared to promote shoot weight of the young plants that were sampled approximately 8 weeks after sowing. Seedling growth enhancement has been associated with some isolates of pathogenic *Fusarium* species in previous report, even though such isolates were also capable of producing root rot symptoms (Ocamb et al. 2002). The plant growth promotion by *F. proliferatum* in our studies is likely due to its ability to produce gibberellins, as this species is well known for producing this plant hormone mimic (Rim et al. 2005).

Table 5. Raw shoot weights of 'Duet' wheat eight weeks after sowing in two replicated greenhouse studies

***Fusarium* isolate[z]**	**Exp. 1 - shoot weight (g)[y]**	**Exp. 2 - shoot weight (g)[y]**
nontreated	5 cd	2 e
Fprol 3-09	7 ab	10 b
Fprol 51-84	8 a	13 a
Fprol 03-20	8 a	9 d
Fprol 03-76	6 cd	9 d
Fprol 02-35	5 d	11 b
Fprol 01-52	6 bc	13 a

[z] Strains of *Fusarium proliferatum* obtained from wheat seed that were used to inoculate pots of wheat in the greenhouse.

[y] Means within the column followed by the same letter are not significantly different at $P = 0.05$ by Fisher's F-protected least significant difference (LSD) test. $R^2 = 0.86$.

BENEFITS AND IMPACT: Our greenhouse studies clearly demonstrate that *F. proliferatum* can incite root disease on wheat when this fungus is present in the soil, but damage at 8 weeks after planting was limited to the seedling plant portions below ground (seminal roots and the mesocotyl). We did not see a reduction in germination with *F. proliferatum* as was reported by Conner et al. (1996), but the temperatures used for the reported studies where germination was impacted were kept at a much cooler temperature (50ºF) than the conditions in the OSU greenhouse (65ºF days/55ºF nights), and we did not apply the *Fusarium* to the seed but rather to the soil in our greenhouse studies. In addition, *F. proliferatum* may promote shoot growth in young wheat plants. So we would consider this a weaker pathogen of wheat in the larger view of pathogenic *Fusarium* species, based on these short-term studies that we conducted in the greenhouse. However, the presence of *F. proliferatum* may enable more aggressive *Fusarium* species to cohabitate on wheat roots, allowing greater ingress by the more pathogenic *Fusarium* strains into wheat roots, as Ocamb & Miller have demonstrated in sweet corn. The sub-lethal effect on roots by weakly pathogenic fungi causes damage to root tissues, and the wounding facilitates easier entry by other *Fusarium* isolates.

Wheat seeds that were infested with *F. proliferatum* in our laboratory assays did not have obvious symptoms characteristic of black point disease (kernel smudge), so this fungus can be present on asymptomatic kernels. *Fusarium proliferatum* was prominent on seed in three of the

six wheat varieties we examined, however, and the presence of this fungus on wheat kernels can result in the formation of mycotoxins, including fumonisins. Our findings raise additional questions as to whether mycotoxins are present on asymptomatic wheat kernels and whether the seedborne or soilborne presence of *F. proliferatum* can enable more aggressive isolates of additional *Fusarium* species to have an easier ingress into wheat plants.

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