# Fusarium spp. as Colonists and Potential Pathogens in Root Zones of Grassland Plants

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ABSTRACT

Chlamydospore-forming *Fusarium* spp. were major midsummer fungal isolates from outer feeder-root zones of representative plants of the western shortgrass plains. Nonfusarial parasites were principal fungal isolates only from inner root tissues of plants in cultivated soils.

Isolates of *F. roseum* were most frequently encountered in gramineous plants, followed in descending order by *F. solani* and *F. oxysporum*. Isolates of *F. solani* were most abundant in sugar beet root zones, followed by *F. roseum* and *F. oxysporum*. Potentially pathogenic forms of *F. roseum* and *F. solani* may be present in the rhizospheres of nonhost grassland plants.

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*Additional key words:* rhizoplane, *Pyrenochaeta, Helminthosporium.*

Since the pioneer studies of Reinking & Manns (15), much work has been done on *Fusarium* populations in soils (1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 19, 26). The most significant research followed the investigations of Nash et al. (8) and Nash & Snyder (9, 10), who developed a selective medium for isolation of *Fusarium* spp. from soils. The few reports on fungal colonization of normal root systems indicate that *Fusarium* spp. are major root inhabitants (14, 22, 23).

Midsummer isolations from the feeder roots of sugar beet (*Beta vulgaris* L.) and barley (*Hordeum vulgare* L.) in the western shortgrass plains for 6 years showed a uniformity of fungal rootlet colonization dominated by *Fusarium* spp. (6). Based on these observations, work was conducted to determine summer fungal colonization of feeder-root zones of representative cultivated and noncultivated plants. Feeder-root zones are considered to consist of four contiguous regions: (i) the inner cortical and vascular tissue; (ii) the feeder-root surface or rhizoplane; (iii) the feeder-root surface plus adhering soil or rhizosphere; and (iv) the free adjoining or outer soil (5). It was hoped that results would increase basic knowledge of soil-borne pathogens of this area.

MATERIALS AND METHODS.—*Plants utilized.*—Cultivated plants used were: barley, *Hordeum vulgare* L.; sugar beet, *Beta vulgaris* L.; maize, *Zea mays* L.; and oat, *Avena sativa* L. Native and introduced grasses from noncultivated soils used were: smooth brome, *Bromus inermis* Leyss.; Japanese chess, *B. japonicus* Thunb.; crested wheatgrass, *Agropyron cristatum* (L.) Gaertn.; western wheatgrass, *A. smithii* Rydb.; blue grama, *Bouteloua gracilis* (H.B.K.) Lag. ex Steud.; side-oats grama, *B. curtipendula* (Michx.) Torr.; Kentucky bluegrass, *Poa pratensis* L.; annual bluegrass, *P. annua* L.; and buffalo grass, *Buchloë dactyloides* (Nutt.) Engelm. Volunteer grasses from cultivated soils used were: green bristlegrass, *Setaria viridis* (L.) Beauv.; and yellow bristlegrass, *S. lutescens* (Weigel) Hubb. Grasses used from both cultivated and noncultivated soils were: slender wheatgrass, *Agropyron trachycaulum* (Link) Malte; quackgrass, *A. repens* (L.) Beauv.; and downy brome, *Bromus tectorum* L. All plants were obtained from Fort Collins and Larimer County (Colorado) loam soils (21) on the western edge of the shortgrass plains at an altitude of ca. 1,524 m (5,000 ft).

*Isolations from feeder-root zones.*—Plants with roots and soil were removed to depths of 10-20 cm from normal habitats during midsummer (15 June – 1 August) for 3 successive years. Fungal colonizations of outer soil zones were appraised by direct plating of soil 0.5-1.5 cm from surfaces of feeder roots, using a modification of Warcup's method (24). Crushed air-dried soil was screened to remove organic debris and to reduce particle diameters to 0.1-0.5 mm, and soil samples (0.05 g) were uniformly dispersed on the surface of nutrient agar in petri dishes.

To estimate colonization of rhizospheres, rootlets were freed of excess soil by gentle tapping, cut into 5-mm sections, and plated without washing. Colonization of rhizoplanes was assayed by plating washed, rinsed, and dried root sections (5 min agitation in warm 0.5% aqueous sodium lauryl sulfate, followed by a 15-min rinse in running tap water, with a final rinse in sterile water and drying on sterile filter paper). Fungal invasion of root tissues was determined by plating washed, surface-disinfested (30 sec in 0.1% $HgCl_2$), and rinsed rootlet sections.

*Media employed.*—Standard 2% potato-dextrose agar (PDA) or 2% water agar was used in initial isolations. Later, when fungal patterns were apparent, a modified Nash-Snyder medium (9) was employed with the following composition: 1.5% peptone, 0.1% $KH_2PO_4$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% pentachloronitrobenzene (75% WP), 300 μg/ml streptomycin sulfate, 250 μg/ml syncillin (soluble penicillin), and 2% agar.

*Method of handling cultures.*—Culture dishes were

incubated at room temp (20-24 C) for 48-96 hr. Fungal colonies from soil particles were counted with the aid of a stereomicroscope, using grids on bottoms of petri dishes.

Where the Nash-Snyder medium was used, developing colonies were transferred (macroconidia) to 2% PDA to permit normal colony development and formation of conidia and chlamydospores. Identifications of *Fusarium* spp. were made following the Snyder-Hansen system (16, 17, 18, 20).

RESULTS.—*Preliminary.*—Initially, washed but not surface-sterilized feeder roots from field-grown sugar beet (1,035 samples) were plated on 2% water agar and PDA. One or more fungal colonies were obtained from 87% of these rootlets. Of this number, 56% showed colonies of *Fusarium* spp., 24% produced growths of *Fusarium* spp. and *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, & Larson, and 7% yielded a miscellaneous group of fungi dominated by *Pythium* spp., *Phytophthora* spp., *Mortierella* spp., *Rhizoctonia solani* Kuehn, *Phoma betae* Frank, and *Actinomucor repens* Schostak. The high incidence of *P. terrestris* was unexpected, there being no record of onion (*Allium cepa* L.), the major host of the parasite in this soil. Simultaneous isolations from barley (*H. vulgare*) growing in an adjoining field also showed that *Fusarium* spp. were dominant fungal root colonists, followed by *Pythium* spp., *Pyrenochaeta terrestris*, and *Helminthosporium sativum* P., K., & B. Isolations were made throughout the remainder of the summer from feeder roots of oat, barley, maize, and sugar beet, major crop plants of the area. *Fusarium* spp. seldom fell below 50% of the total isolates.

*Selectivity of a modified Nash-Snyder medium.*—Since *Fusarium* spp. appeared to be the dominant fungi in the rhizoplane and cortical zones of the feeder roots of representative major crop plants investigated, a modified Nash-Snyder medium (9) was chosen for further isolation work. The basic medium reportedly favors *Fusarium* spp. by retarding growth competitors such as most Zygomycetes, Streptomycetes, and Eubacteria (9, 10, 12).

To determine the selectivity of the modified medium (see MATERIALS AND METHODS), 75 organisms, representative of the local soil microflora, were transferred to petri dishes of this medium and standard 2% PDA. Plates were incubated at 22-26 C for 10 days. The modified Nash-Snyder medium prevented or markedly reduced growth of Basidiomycetes, Eubacteria, Actinomycetes, and most of the imperfect fungi tested. Zygomycetes, with the exceptions of the genera *Mortierella* and *Actinomucor*, were also adversely affected. Growth of Oomycetes (*Pythiaceae*) and the nine type species of *Fusarium* (20) was not suppressed.

*Fungal colonization patterns in the root zones of two major cultivated plants.*—Isolations were made from the root zones of barley and sugar beet growing in adjoining fields. The results shown in Fig. 1-A and 1-B are based on a total of 600 randomly selected and identified isolates, and give the frequencies of fungi isolated for each root zone.

*Fusarium* spp. were the major fungi obtained (62-87% of the isolates) from all but the inner root zones of barley, where nonfusarial fungi dominated, constituting 65% of the isolates (Fig. 1-A). *Fusarium roseum* (Link) emend. Snyd. & Hans., the principal fungus found, progressively declined from the outer (63%) to the inner zones (26% of the isolates). Conversely, there was an increasing incidence of nonfusarial fungi from the outer (13%) to inner zones (65% of the isolates). Nonfusarial isolates included potential parasites such as *Pythium* spp., *R. solani, H. sativum,* and *P. terrestris,* and common saprophytes such as *Mortierella* spp., *Cephalosporium* spp., and *Trichoderma viride* Pers. Saprobes were usually confined to external zones; parasites to inner zones. *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. was of secondary prevalence in barley, showing decreasing isolation frequencies from outer soil and rhizoplane zones (19 and 24%, respectively) to inner root tissues (7%). *Fusarium oxysporum* (Schlecht.) emend. Snyd. & Hans. was least prevalent (2-9%) of the isolates.

*Fusarium* spp. comprised 58-78% of the isolates and dominated in all root zones of sugar beet (Fig. 1-B). The most common fungus, *F. solani,* showed a declining isolation frequency from the outer soil (54%) to the inner root zones (33%). Isolates of *F. roseum* varied from 20% in the outer soil zone to 31% in the rhizoplane, whereas *F. oxysporum* again was of minor frequency in all zones (2-10%). With two differences, the same nonfusarial fungi found in barley were present in the inner root zones of sugar beet (42% of the isolates). These disparities were the absence of *Helminthosporium* spp. present in barley, and the presence of *Phytophthora* spp., absent in barley.

*Fungal colonization of root zones of representative grasses.*—Isolations were made from the root zones of 13 species of six representative gramineous genera. Grasses were grouped according to their occurrences in cultivated and noncultivated soils (see MATERIALS AND METHODS). Attempts to compare data on a taxonomic basis were abandoned since no consistent differences were found. A total of 900 randomly selected isolates obtained from the four soil-root zones was identified in this study.

Figure 1-C shows that *Fusarium* spp. dominated in all feeder-root zones of representative volunteer grasses in cultivated soils (68-94% of the isolates). *Fusarium roseum*, the major fungus found, ranged from 71% of the total isolates in the outer soil to 40% in the inner root zones. *Fusarium solani* was secondary in prevalence (15-31% of the isolates), followed by *F. oxysporum* (8-20%), neither species showing a gradient trend. Nonfusarial fungi were of minor occurrence, although revealing a progressively rising incidence from outer (6%) to inner zones (32% of the isolates).

*Fusarium* spp., dominated by *F. roseum*, were the principal fungi in all four feeder-root zones (82-89%) of native and introduced grasses in noncultivated soils (Fig. 1-D). *Fusarium roseum* and nonfusarial fungi showed decreasing gradients from outer to inner

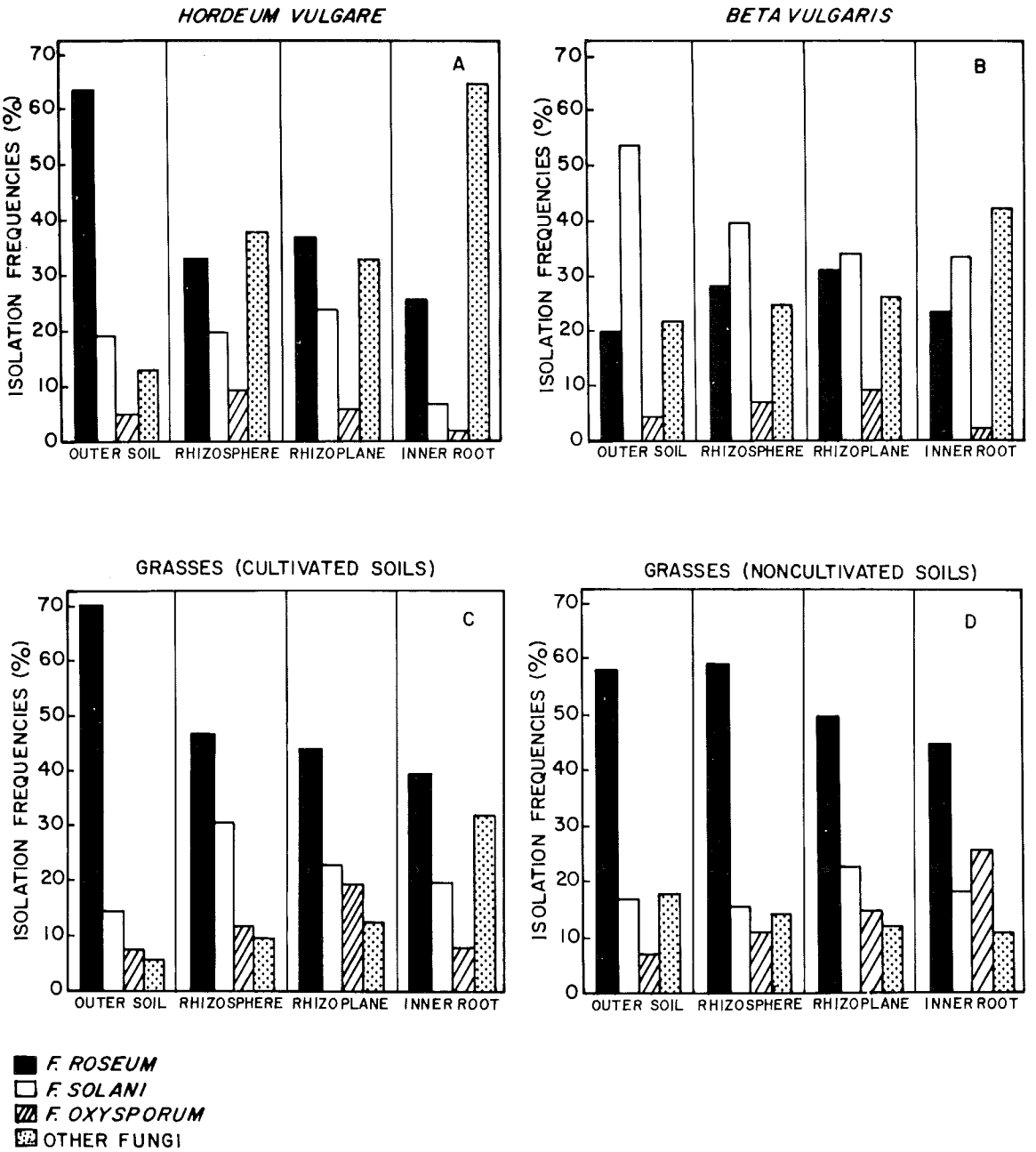


**Fig. 1.** Isolation frequencies of *Fusarium* spp. and other fungi within feeder-root zones of representative plants from cultivated and noncultivated soils of the western shortgrass plains region.

zones (58-45% and 18-11%, respectively), whereas a rising trend was indicated by *F. oxysporum* (7-26%). *Fusarium solani* showed no gradient (16-23%).

*Chlamydospore- versus nonchlamydospore-forming Fusarium spp. in the root zones of all plants investigated.*—According to the Snyder-Hansen system of classification, the genus *Fusarium* comprises nine basic species (16, 17, 18, 20). Five of these species were isolated in this study (Table 1). Of 1,921 *Fusarium* colonies isolated, 53.2% were *F. roseum*, 33.6% were *F. solani*, and 12.3% were *F. oxysporum*. Other isolates were *F. tricinctum* (Corda) emend. Snyd. & Hans. (0.8%) and *F. moniliforme* (Sheld.) emend. Snyd. & Hans. (0.1%), the latter being the only nonchlamydospore-forming species found.

*Pathogen reservoirs in the rhizospheres of cultivated and noncultivated plants.*—Prelimary work was conducted to determine whether root zones of non-

diseased plants of the region might harbor pathogenic forms of *Fusarium* spp. Eight random but different *F. solani* isolates originally obtained from the rhizospheres of sugar beet, and 12 *F. roseum* isolates from the rhizospheres of the noncultivated grasses *Agropyron repens* and *A. trachycaulum*, were used to infest separate lots of screened and autoclaved field soil in plastic pots (7.5 cm deep × 11.5 cm in diam) at inoculum density rates of approximately 10,000 macroconidia/g of soil. Soils infested with *F. roseum* isolates were planted with barley (Betzes cultivar); lots infested with *F. solani* isolates were planted with bush bean (*Phaseolus vulgaris* L.). The results of this test showed that one *F. roseum* isolate of the Culmorum type from *A. trachycaulum* was highly pathogenic to barley seedlings, and two *F. solani* isolates from *B. vulgaris* were moderately pathogenic to bush bean seedlings.

DISCUSSION.—Warcup (24, 25) emphasized that the apparent fungal composition of a soil depends on the isolation methods. Isolations from root zones also will reflect the bias imposed by the methods. The dry-particulate isolation technique used in this work in the midsummer bioassay of the outer soil and rhizosphere zones of plants favors sterile mycelium-forming or dormant spore-forming (e.g., chlamydospores) fungi. We preferred this method to the dilution-plate technique which is selective for those fungi readily producing conidia or their equivalents. The writer knows of no isolation methods which will accurately segregate the fungi in each of the designated biologically overlapping feeder-root zones. Nevertheless, the techniques used in this study appeared to give consistent and interpretable, albeit restricted, results; the resulting picture revealing but a facet of that existing in nature.

The indicated fungal compositions of the four root zones for all plants studied were similar. Preliminary platings on nonspecific media revealed that *Fusarium* spp. were the major fungi in the combined rhizoplane-inner root zones of sugar beet and barley. Later results, using the Nash-Snyder selective medium, showed the expected dominance of *Fusarium* spp. in all except the inner root zones of these plants, where nonfusarial fungi were preponderant. Grasses, irrespective of source, showed a predominance of *Fusarium* spp. in all feeder-root zones. However, the highest percentage of nonfusarial fungi was found in the inner root zones of grasses from cultivated soils. In all gramineous plants, the principal fungal species in every zone was *F. roseum*. This finding agrees with the report of Snyder & Nash (19), who found a preponderance of *F. roseum* in Rothamsted cereal soils. A host effect was observed in sugar beet in which *F. solani* replaced *F. roseum* as the major species in each root zone. Although *F. oxysporum* was present in the root zones of all plants investigated, it was secondary in prevalence. These observations differ from results of root isolations in other regions, where it was found that *F. oxysporum* was the dominant *Fusarium* species (22, 23).

Principal fungal colonists, as shown by the methods used, were chlamydospore-forming species of *Fusarium*, 87% of these being in the *F. roseum-F. solani* category. Invasion of senescent and moribund feeder roots by saprophytic and weakly parasitic clones of these soil-inhabiting species should be a logical sequence of their colonization of outer feeder-root zones. The absence of isolates of the nonchlamydospore-forming *Fusarium nivale* (Fr.) Snyd. & Hans. confirms that this fungus is neither a soil nor root inhabitant (19); being instead a cool-weather pathogen of gramineous crowns and leaves. Finally, the presence of forms of *F. roseum* (Culmorum) and *F. solani* (presumably *F. solani* f. sp. *phaseoli*) in the rhizospheres of healthy grassland plants, potentially pathogenic to crop plants, is not surprising. *Fusarium roseum* and *F. solani* may play a more important role than hitherto believed in the subterranean pathology of plants of the western shortgrass plains.

TABLE 1. Percentages of chlamydospore- and nonchlamydospore-forming *Fusarium* spp. isolated from feeder-root zones of representative plants of the western shortgrass plains[a]

| Species | Frequencies, % | Chlamydospores[b] |
|---|---|---|
| *F. roseum* | 53.2 | + |
| *F. solani* | 33.6 | + |
| *F. oxysporum* | 12.3 | + |
| *F. tricinctum* | 0.8 | + |
| *F. moniliforme* | 0.1[c] | – |

[a]Based on a total of 1,921 *Fusarium* isolates.
[b]Presence or absence of chlamydospores (Snyder-Hansen system of classification).
[c]Isolated only from cortical zones of *Bromus inermis*.

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