Fungi Associated with Common Root Rot of Winter Wheat in Colorado and Wyoming

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ABSTRACT

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Winter wheat plants were sampled during two growing seasons in Colorado and Wyoming to identify fungi associated with common root rot. Isolates derived from diseased wheat were grown in pure culture and screened for pathogenicity. Of 852 fungal isolates tested, 408 were pathogenic to seedling wheat and most were identified. Of the screened isolates, 139 (34%) were *Bipolaris sorokiniana* and 225 (55%) were *Fusarium* species, of which 113 (28%) were *F. roseum* 'Acuminatum.' In fall and early spring, *B. sorokiniana* was usually the fungus most frequently isolated. Both *B. sorokiniana* and *F. roseum* 'Acuminatum' were usually isolated from diseased tissues from early summer to crop maturity. *B. sorokiniana* and *F. roseum* 'Acuminatum' are considered primary components of the common root rot complex in Colorado and Wyoming.

Additional key words: foot rot

Common root rot is a well-known major disease of wheat (9,27). Typical symptoms of common root rot include necrosis of the roots, subcrown internode, crown, culms, and lower leaf sheaths of affected plants (2,12,15). In addition, plants may show premature senescence and produce sterile or partially filled white heads (19). Early infections by the causal fungi often result in seedling death (27).

The etiology of common root rot is complex and varies regionally. *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. is considered the major incitant of common root rot in the Canadian prairies (8,15,26), North Dakota (20), and Queensland, Australia (13). Studies in other areas of Australia (2), East Africa (14), California (12,16), and Nebraska (6) indicate *B. sorokiniana, Fusarium roseum* Link emend. Snyd. & Hans. 'Culmorum' (=*F. culmorum* W. G. Smith), and *F. roseum* 'Graminearum' (=*F. graminearum* Schwabe) are the major pathogens. In the Pacific Northwest, foot and root rot of wheat is caused by *F. roseum* 'Culmorum' and 'Graminearum' (3,4).

Common root rot of fall-sown wheat is often associated with plant stress caused by cold dry winters and low soil moisture in summer (6). Such conditions often occur in the wheat-producing areas of Colorado and Wyoming where the disease is considered a major yield constraint (10). In 1979, reductions in fall-sown wheat stands of 20–55% were attributed to common root rot in some areas of Nebraska (24). Similar stand reductions were also observed in areas of Colorado and Wyoming and initial isolations from diseased wheat demonstrated the presence of *B. sorokiniana*. *Fusarium* spp. other than *F. roseum* 'Culmorum' or 'Graminearum,' however, were also isolated. Because of the regional importance of the disease and the unusual spectrum of species recovered in preliminary isolations, studies were initiated to identify the fungi associated with common root rot of winter wheat in the major wheat-producing areas of Colorado and Wyoming.

Some of the information presented herein is a portion of the third author's M.S. thesis.

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MATERIALS AND METHODS

Isolation and maintenance of cultures. Diseased winter wheat was collected from 46 fields in Colorado and Wyoming between September 1978 and July 1980. Plants at the seedling stage of growth through maturity were sampled. In addition, diseased wheat samples received by the Colorado and Wyoming Plant Disease clinics were included in the study.

Plants were washed thoroughly and sections of roots, subcrown internodes, crowns, lower culms, and leaf sheaths showing symptoms of common root rot were surface-disinfested in 0.5% NaOCl for 30 sec or in a mixture of 5.25% NaOCl and 95% ethanol (1:2, v/v) for 10 sec. Outer tissues were removed and discarded and the remaining pieces plated on potato-dextrose agar (PDA) to which streptomycin sulfate (50 μg/ml) was added if bacterial contamination was a problem. Culture plates were incubated under ambient laboratory conditions for 1–2 wk. In the Colorado studies, Nash and Snyder's PCNB agar (23) and dextrose-peptone yeast agar (23) were also used for isolations.

Cultures of *Fusarium* spp. were transferred to carnation leaf agar (CLA) (22) and subsequently single-spored to CLA or PDA slants for storage. Fungi other than *Fusarium* spp. were transferred directly to PDA slants for storage. In the Colorado studies, only *Fusarium* and *Bipolaris* spp. were stored for further tests. All cultures were stored at 5 C.

Pathogenicity tests. Unless otherwise stated, the following procedures were used: Wheat seed (cultivar Scout) was treated for 10 min in 0.52% NaOCl. Seedlings were grown in test tubes measuring 1.5 × 15 cm to which were added 5-mm^2 PDA blocks supporting growth of the fungus to be tested. In Colorado, tubes were partially filled with sterile vermiculite and the inoculum block was placed 1 cm below the seed. If contamination was observed, the procedure was repeated. In Wyoming, 2-day-old seedlings germinated on PDA and visually free of fungal contaminants were placed in tubes containing a folded piece of sterile filter paper. Inoculum blocks were placed next to the seedling. Three replicates were used for each isolate.

Tubes were watered as needed and incubated for 2–3 wk. Seedlings then were removed from the tubes and rated for disease severity on a scale of 0–3 representing no, slight, moderate, and severe discoloration of the coleoptile or roots. All isolates receiving a pathogenicity rating of two or greater were selected for identification.

Identification. Isolates of *Fusarium* spp. were transferred from stock cultures to CLA and PDA and the keys of Booth (1) and Toussoun and Nelson (22) were used as aids in identification. Representative cultures of some *Fusarium* spp. were sent to the Fusarium Research Center, Pennsylvania State University,

for confirmation of identifications. Fungi other than *Fusarium* spp. were identified using the keys of Sprague (19) and Ellis (5).

RESULTS

A total of 852 fungal isolates was rated for pathogenicity; of these, 408 received ratings of two or greater and were identified. *B. sorokiniana* was the most frequently isolated species, representing 34% of the fungi identified (Table 1). Of the *Fusarium* spp. identified, *F. roseum* 'Acuminatum' (=*F. acuminatum* Ell. & Ev.) was the most prevalent (28%).

Other *Fusarium* spp. and their frequency of isolation are listed in Table 1; with the exception of *F. roseum* 'Acuminatum,' none exceeded 6% in frequency of isolation. The remaining 12% of isolates consisted of *Hendersonia crastophila* Sacc., *Nigrospora sphaerica* (Sacc.) Mason, *Curvularia inaequalis* (Shear) Boed., and nonsporulating (unidentified) fungi (Table 1).

Most isolates of *B. sorokiniana* were recovered from root, subcrown internode, and crown tissues. Generally, this fungus was recovered from diseased plants throughout the growing season (fall through summer). Most isolates of *F. roseum* 'Acuminatum' were recovered from crown tissues, although the fungus was also recovered from subcrown internode and culm tissues. Most isolates of *F. roseum* 'Acuminatum' (about 87%) were recovered from diseased plants collected during the spring and summer.

DISCUSSION

B. sorokiniana is a well-known component of the common root rot complex in many cereal-producing areas of the United States and Canada and occurs on a wide range of grass hosts (19). It appears to be the primary incitant of common root rot in the northern Great Plains and Canada (17,19,25). Therefore, it is not surprising that *B. sorokiniana* was the fungus most frequently isolated in Colorado and Wyoming.

In addition to *B. sorokiniana*, other fungi usually named as additional components of the common root rot complex are *F. roseum* 'Culmorum' and 'Graminearum' (2,6,13,15,17); however, we isolated these fungi very infrequently (Table 1). *F. roseum* 'Culmorum' has not been found in abundance in the northern Great Plains (19) so it was not surprising that we recovered this fungus infrequently. In these studies, the *Fusarium* sp. most frequently associated with common root rot and *B. sorokiniana* was *F. roseum* 'Acuminatum' (Table 1).

Although *F. roseum* 'Acuminatum' has been listed occasionally as a component of the common root rot complex in some areas (7,19,21), this fungus has not been considered an important pathogen of cereals (1,19). Sprague (18) considered it to be a secondary parasite of grasses and of slight importance on wheat. Therefore, it was somewhat surprising to find *F. roseum* 'Acuminatum' consistently associated with common root rot. This association, however, may be more prevalent than published reports indicate. In this regard, Sprague (19) considered *F. roseum* 'Culmorum' to be a catchall for any pink- or carmine-tinted *Fusarium* spp. found in the United States and speculated that often *F. roseum* 'Acuminatum' has been mistakenly called 'Culmorum.' Considering the similar gross cultural characteristics of these two fungi, we believe this is very probable.

The pathogenicity tests employed in these studies were not intended to simulate natural soil conditions. Quite the contrary, they were deliberately designed to provide for maximum expression of pathogenicity for the following reasons: Early in these studies, it became apparent that using standard isolation techniques often resulted in the frequent recovery of fungi normally considered saprophytic. This was especially true of samples obtained from wheat showing severe symptoms of common root rot.

Because the etiology of common root rot is widely considered to be a complex of *B. sorokiniana* and other fungi, usually *Fusarium* spp., this presented a dilemma. Identification of all fungi associated with diseased tissues, especially those recovered from severely affected plants, might result in isolation frequencies biased for saprophytes. On the other hand, it has been demonstrated that *B. sorokiniana* can predispose plants to severe infections by fungi normally considered weak pathogens (11). Thus, ignoring isolates reported to be weak pathogens could result in failure to identify potentially important etiological complexes. To ameliorate this dilemma, a compromise approach was selected. Isolates were screened in pathogenicity tests intentionally designed to exclude only those fungi incapable of producing at least moderate symptoms under optimum conditions for disease development. This seemed a reasonable approach to ensure inclusion of any potential members of the disease complex. It is interesting to note that all *Fusarium* spp. commonly cited as members of the common root rot complex were selected by this method, albeit infrequently (Table 1).

Because of the nature of these tests, however, little can be said concerning the pathogenicity of *F. roseum* 'Acuminatum' (or for that matter, *B. sorokiniana*) as it occurs in the field. Nevertheless, the consistent association of *F. roseum* 'Acuminatum' with diseased plant tissues leads us to believe that its importance as a pathogen, although probably secondary, may be underestimated.

Our reasoning is as follows: In the fall, plants were usually parasitized exclusively by *B. sorokiniana*. In the spring and summer, plants were often infected by both *B. sorokiniana* and *F. roseum* 'Acuminatum.' Others (21) have shown that prepossession of host tissues by *B. sorokiniana* does not exclude invasion by *F. roseum* 'Acuminatum' or 'Culmorum.' Indeed, Ludwig et al (11) demonstrated that *B. sorokiniana* or its culture filtrates predispose barley roots to severe infections by fungi normally considered weakly pathogenic. We therefore hypothesize the following sequence of events in the common root rot complex as it occurs in Colorado and Wyoming: *B. sorokiniana* infects wheat during the fall or early spring. This infection predisposes roots to invasion by *F. roseum* 'Acuminatum' and perhaps, to a lesser extent, other fungi normally not considered primary pathogens of wheat (Table 1).

It seems clear that our findings and those of others (11,21) support such a scheme. What is not clear, however, is of what importance additional infections by *F. roseum* 'Acuminatum' are over initial

Table 1. Fungi associated with common root rot of wheat in Colorado and Wyoming

Fungus	No. of isolates Colorado/Wyoming	Total no. of isolates	Percent of total
Bipolaris sorokiniana	80/59	139	34
Fusarium roseum 'Acuminatum'	73/40	113	28
F. roseum 'Sambucinum'	21/4	25	6
Hendersonia crastophila	21[a]	21	5
F. solani	2/17	19	5
F. roseum 'Avenaceum'	15/1	16	4
F. nivale	4/11	15	4
F. roseum 'Graminearum'	10/2	12	3
F. roseum 'Equiseti'	2/8	10	2
F. oxysporum	1/8	9	2
F. roseum 'Culmorum'	1/2	3	<1
F. tricintum	0/3	3	<1
Curvularia inaequalis	3[a]	3	<1
Nigrospora sphaerica	3[a]	1	<1
Nonsporulating fungi	8/11	19	5
Totals	217/191	408	

[a] Included in Wyoming studies only.

infections by *B. sorokiniana*. Research is currently in progress to determine this.

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