Association of *Bipolaris sorokiniana, Fusarium graminearum* Group 2, and *F. culmorum* on Spring Wheat Differing in Severity of Common Root Rot

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ABSTRACT

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Forty fields of spring wheat (wheat planted after wheat) in the early-milk to ripe stages were sampled in northwestern Minnesota during 1985–1987. Subcrown internode ratings (0–3 scale, where 0 = healthy, 3 = ≥50% discolored) during the 3 yr averaged 0.9. In 1985 and 1986, *Bipolaris sorokiniana* was isolated from significantly fewer subcrown internodes in disease classes 0 and 1 than in disease classes 2 and 3; in 1987, recovery of this fungus was the same for all disease classes. Isolation of *B. sorokiniana* from crowns of the same plants in classes 0, 1, 2, and 3 and from plants with no subcrown internodes did not differ significantly in each of the 3 yr and averaged 76%. *Fusarium graminearum* group 2 was recovered from 7% of the subcrown internodes and 9% of the crowns, whereas *F. culmorum* was isolated from 2% of the subcrown internodes and 4% of the crowns. Although subcrown internode ratings were low, *B. sorokiniana, F. graminearum* group 2, and *F. culmorum* were isolated from symptomless and lesioned subcrown internodes and crowns of roots in all disease classes.

Additional keywords: *Cochliobolus sativus, Gibberella zeae, Triticum aestivum*

Several soilborne pathogens of wheat (*Triticum aestivum* L.) cause common root rot, with necrosis of basal stems, crowns, subcrown internodes, and roots. The disease involves a complex of fungi, but the most prevalent pathogen is *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. (syns. *Helminthosporium sativum* P.K. & B., *H. sorokinianum* Sacc. ex Sorok.; teleomorph *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dast.) (21). *Fusarium graminearum* Schwabe and *F. culmorum* (W.G. Smith) Sacc., alone or together with *B. sorokiniana*, also have been obtained from lesioned tissues (18). Other pathogens in the disease complex include: *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., *F. crookwellense* Burgess, Nelson, & Toussoun, and *F. poae* (Peck) Wollenw. (21). Fungi causing common root rot take on additional importance as pathogens that cause seedling blight (seedborne and soilborne inoculum), leaf spots, and kernel diseases of wheat.

By anthesis, most plants in a field may be infected (18), but disease symptoms often are inconspicuous, especially if soil moisture is not limited. Infections of subcrown internodes and crowns usually remain in the chronic (common root rot) stage, where the subcrown internode is a visual indicator of disease (10,18). The more severe, acute phase of common root rot (dryland foot rot) can occur under very low plant water potentials (3). Infected plants usually occur randomly or in irregular patches and appear stunted and chlorotic; yield and seed quality are reduced.

Common root rot is widely documented in the Great Plains of North America (10,18,21), but there is no information documenting the importance of the disease, or the relative prevalence of the fungi causing it, in northwestern Minnesota. About 630,000 ha of spring wheat are grown annually in northwestern Minnesota, representing 60% of the crop produced in the state (Minnesota Agricultural Statistics Service, St. Paul). Information gained from this study will be a basis for future research on root diseases of spring wheat in the region.

The objectives of this study were to evaluate the severity of common root rot on spring wheat grown in northwestern Minnesota and to determine the relative incidence of *B. sorokiniana* and *Fusarium* species associated with plants differing in severity of common root rot. Preliminary results have been reported (23).

MATERIALS AND METHODS

Fields of spring wheat at the early-milk to ripe maturity stages (72–91 growth stages) (26) were sampled from 11 counties in northwestern Minnesota: 11 fields during 23–26 July 1985 (early milk to early dough), 18 fields during 23 July–8 August 1986 (late milk to medium dough), and 11 fields during 13–24 July 1987 (early dough to ripe). One hundred plants were randomly collected on a diagonal across each field. The fields had been planted to wheat the previous season. All producers but one knew the cultivar planted, and 81% had planted the cultivar Marshall. Marshall had also been planted in 75% of these fields the previous season.

Roots were thoroughly washed, and the subcrown internodes were examined and visually rated for lesions on a 0–3 scale, where 0 = healthy, 1 = 1–25% of the area lesioned (mild), 2 = 26–50% lesioned (moderate), and 3 = ≥50% lesioned (severe) (10). Subcrown internode ratings were calculated by summing the products of the number of plants in each category multiplied by the disease category value, then dividing the sum by the number of plants sampled.

To evaluate incidence of common root rot fungi, whole subcrown internodes were separated according to disease class, surface-treated in 0.5% NaOCl for 30 sec, rinsed twice in sterile distilled water, drained on paper towels, and placed on potato-dextrose agar (PDA).

Crowns from plants in each disease class (based on the subcrown internode rating) were trimmed to within 2.5–3 cm of the stem base, cut in half, and separated. This tissue was then surface-treated in 1% NaOCl for 1 min, rinsed twice in sterile distilled water, and drained on paper towels. One set of halved crowns was placed on a medium selective for *B. sorokiniana* (15) and the other half was cultured on chlortetracycline-supplemented pentachloronitrobenzene (PCNB) agar, a medium selective for *Fusarium* (12).

All cultures were grown 48.5 cm below a combination of fluorescent lights (three General Electric or Sylvania 40W tubes) and black lights (two Sylvania 40W, BLB series) for a 12-hr photoperiod for 1–2 wk. Cultures growing from subcrown internodes were microscopically examined and identified as *B. sorokiniana* or to *Fusarium* species (13).

Cultures growing from crowns on the medium selective for *B. sorokiniana* were confirmed by microscopic examination

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of conidia; if characteristic growth of the fungus was obscured by other microorganisms, the surface of the crown was scraped with a transfer needle and removed conidia were examined microscopically. All *Fusarium* cultures growing from crowns onto PCNB agar were transferred to PDA and incubated for 10–14 days. Cultures that were pink to red (and characteristic of species in the Sections Arthrosporiella, Discolor, Gibbosum, Roseum, and Sporotrichiella) were transferred to carnation leaf agar (CLA) and also hyphal-tipped to homemade, acidified potato-dextrose agar (13). After 10–14 days, *Fusarium* species were identified.

Identification of group 1 and group 2 populations of *F. graminearum* cultures from subcrown internodes and crowns were confirmed by growing them on CLA (13). When perithecia of *Gibberella zeae* (Schw.) Petch formed (usually in 3–4 wk), they were microscopically examined for mature ascospores.

Statistical analyses (analysis of variance, mean separations, and correlations) were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). When percent occurrence of *B. sorokiniana* and *F. graminearum* plus *F. culmorum* in each disease class was analyzed, each field was treated as a replicate. Arcsine transformations were performed on percentage data. A significance level of $P = 0.05$ was used in all statistical tests.

RESULTS

Subcrown internode ratings. Among the 4,000 plants evaluated in three seasons, 27% had subcrown internodes in class 0, 22% in 1, 5% in 2, and 9% in 3; the remaining 37% had no subcrown internodes. Nearly all of the fields had plants with no subcrown internodes; incidence per field ranged from 0 to 85% (av. 33%) in 1985, from 8 to 58% (av. 34%) in 1986, and from 3 to 53% (av. 30%) in 1987.

Plants with no subcrown internodes were excluded when subcrown internode ratings were calculated. The ratings among fields for each season were low, ranging from 0.3 to 1.3 (av. 0.7) in 1985, from 0.4 to 1.7 (av. 0.9) in 1986, and from 0.5 to 2.0 (av. 1.1) in 1987.

Common root rot fungi in symptomless subcrown internodes. The lowest recovery of *B. sorokiniana* was from lesion-free subcrown internodes in class 0 (healthy). In 1985 and 1986, *B. sorokiniana* was isolated from significantly fewer ($P = 0.05$) healthy subcrown internodes than lesioned subcrown internodes in classes 1–3 (Fig. 1). In 1987, isolation of the fungus was the same for all subcrown internodes in classes 0–3 (Fig. 1). *B. sorokiniana* was isolated from 18, 34, and 40% of the subcrown internodes in class 0 in 1985, 1986, and 1987, respectively.

F. graminearum and *F. culmorum* were not isolated from subcrown internodes in class 0 in 1985, but these two species combined were isolated from 6 and 17% of symptomless subcrown internodes in 1986 and 1987, respectively. Although data are not shown, occurrences of *F. graminearum* and *F. culmorum* were not significantly different ($P = 0.05$) for subcrown internodes in classes 0–3 during 1986 or 1987.

Common root rot fungi in lesioned subcrown internodes. *B. sorokiniana* was the predominant fungus isolated from subcrown internodes in disease classes 1–3 during the three seasons in all fields. In 1985 and 1986, *B. sorokiniana* was isolated from significantly fewer subcrown internodes in class 1 than in classes 2 and 3, where recovery of this fungus was the same (Fig. 1). During 1987, there were no significant differences in recovery of the fungus from subcrown internodes in classes 1, 2, and 3 (Fig. 1).

F. graminearum and *F. culmorum* combined were isolated only from subcrown internodes in class 3 (2%) during 1985. Occurrences of *F. graminearum* and *F. culmorum* in subcrown internodes in classes 0–3 during 1986 and 1987 are shown in Figure 2. Overall, *F. graminearum* and *F. culmorum* were isolated from 8.5% of the subcrown internodes, and 30% of these internodes were also infected by *B. sorokiniana.*

Fusarium species reported as less virulent pathogens of wheat also were isolated. Over three seasons, *F. avenaceum* was isolated from 1% of the subcrown internodes, *F. acuminatum* from 3%, and *F. poae* from 2%. Other *Fusarium* species isolated included: *F. dimerum* Penzig, *F. equiseti* (Corda) Sacc. sensu Gordon, *F. oxysporum* Schlecht. emend. Snyd. & Hans., *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. sporotrichioides* Sherb., and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun, & Marasas comb. nov.

Other organisms isolated from subcrown internodes. Fungi or bacteria were isolated from nearly all of the subcrown internodes rated as 1, 2, or 3; microorganisms were not isolated from 10% of the subcrown internodes in class 0. Several genera of fungi were isolated from subcrown internodes, especially *Alternaria*, which was isolated from 19, 20, 13, and 10% of the subcrown internodes in classes 0, 1, 2, and 3, respectively. Other fungi isolated included: *Aspergillus, Epicoccum, Nigrospora, Penicillium, Pythium, Trichoderma*, several unidentified dark-spored fungi, and rapid-growing fungi with no conidia. Cultures of AG-5 of *Rhizoctonia solani* Kühn were isolated from 27 subcrown internodes and AG-4, from two.

Common root rot fungi in crowns. Lesions frequently were observed on crowns from plants in each disease class and from crowns of plants with no subcrown internodes. Data are not shown, but isolations of *B. sorokiniana* from crowns of plants with subcrown internodes in classes 0–3 or from crowns of plants with no subcrown internodes were not significantly different ($P = 0.05$) in each of the three seasons. When the three seasons were combined, recovery of *B. sorokiniana* averaged 72, 80, 81, and 80% for classes 0, 1, 2, 3, respectively,

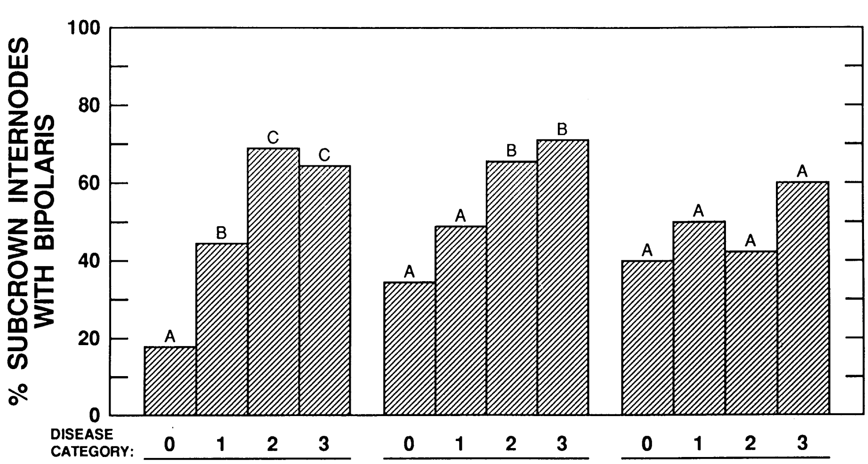

Fig. 1. Percentage of subcrown internodes of spring wheat plants in various disease severity classes from which *Bipolaris sorokiniana* was isolated. Eleven fields were sampled in 1985, 18 in 1986, and 11 in 1987. For each year, bars with the same letter are not statistically different ($P = 0.05$), Student-Newman-Keuls test.

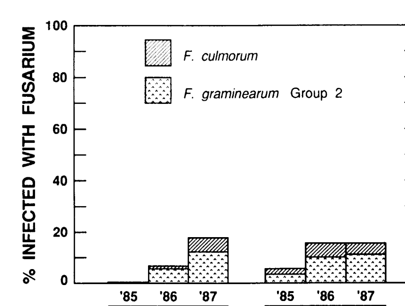

Fig. 2. Percentage of subcrown internodes (SCI) and crowns of spring wheat from which *Fusarium graminearum* group 2 and *F. culmorum* were isolated. Eleven fields were sampled in 1985, 18 in 1986, and 11 in 1987.

and 76% for plants with no subcrown internodes. Overall isolation of the fungus from wheat crowns averaged 75, 77, and 79% in 1985, 1986, and 1987, respectively.

Data are not shown, but isolations of *F. graminearum* and *F. culmorum* from crowns of plants in each disease class and from crowns of plants with no subcrown internodes were not significantly different ($P = 0.05$) in each of the three seasons. Isolation of *F. graminearum* and *F. culmorum* from crowns varied among seasons (Fig. 2).

During the three seasons, *F. avenaceum* was isolated from 3% of the crowns, *F. acuminatum* from 8%, and *F. poae* from 14%. *F. crookwellense* was isolated from a single crown in 1986. Other *Fusarium* species isolated from crowns included the same as those found in subcrown internodes.

Perithecial formation. Of the 517 cultures of *F. graminearum* that were hyphal-tipped, 99.6% produced perithecia of *G. zeae* bearing mature ascospores (= *F. graminearum* group 2). These cultures originated from subcrown internodes and crowns.

Correlation between crown and subcrown internode infections. Correlation coefficients were computed to describe the relationship between the frequency of common root rot fungi isolated from subcrown internodes and crowns (Table 1). A significant correlation occurred between the percent subcrown internodes and crowns infected by *B. sorokiniana* in one of the three seasons. Significant correlations also were found for the percent subcrown internodes and crowns infected by *F. graminearum* and *F. culmorum* in two seasons.

DISCUSSION

Severity of common root rot is notoriously variable because fungi in the disease complex interact strongly with environmental factors, especially moisture. From 1985 to 1987, overall soil moisture was adequate during most of the growing season, which likely favored infection but limited symptom development (21). Fields were sampled in several counties, and rainfall varied among fields. Moreover, a single subcrown internode rating near the end of the growing season fails to consider disease progression over time (19).

Over 25% of the subcrown internodes were in the clean (symptomless) category, but 29% of these tissues were infected by *B. sorokiniana* and 6% by *F. graminearum* and *F. culmorum*. Harding (8) also found that incidence of *B. sorokiniana* was higher than expected on the basis of isolation from lesion-free subcrown internodes. These subcrown internodes may have been infected and sampled before typical lesions developed.

Only 14% of the subcrown internodes were in the moderate to severe disease classes, where plant yields are most likely to be reduced (18). Yield losses were not estimated, but with most roots in the clean to mild disease classes, yield losses would likely be minimal.

Each disease class detected the presence of common root rot fungi, but lesions on subcrown internodes (which may not always be "typical" of common root rot) infected by other fungi may "inflate" rating values. For example, the subcrown internode rating based on visual assessments during 1985–1987 averaged 0.9; a subcrown internode rating calculated on the basis of isolation of *B. sorokiniana, F. graminearum*, and *F. culmorum* in disease classes 1–3 would average 0.5. Whether "inflated" index values are of consequence is uncertain, since many factors affect isolation of fungi. For instance, isolation procedures, culture media, or presence of other fungi may have inhibited growth of common root rot fungi, so their recovery would be conservative. In several instances, obviously lesioned subcrown internodes yielded no fungi.

F. graminearum and *F. culmorum* apparently are effective colonizers of subcrown internodes. Both species usually were isolated without the presence of *B. sorokiniana*. Tinline (16) determined that prepossession of the internode by *B. sorokiniana* did not prevent later infection by fusaria, but that *B. sorokiniana* usually was unable to infect subcrown internodes already infected by *F. culmorum* and *F. acuminatum*. *F. culmorum* also has been shown to be more pathogenic than *B. sorokiniana* to wheat seedlings (20).

Nearly 100% of the isolates of *F. graminearum* isolated from roots were the group 2 population. These results are inconsistent with reports that isolates of *F. graminearum* from wheat roots represent the group 1 population (7,14). Isolates of *F. graminearum* from aboveground plant parts form perithecia (= group 2) (7). It is unknown, however, if perithecia form on wheat root residue in nature. Cultures of *F. graminearum* isolated from cornstalks (24), barley roots (C. E. Windels, *unpublished*), wheat grain (22), and wheat roots indicate that group 2 is the dominant population of this fungus in Minnesota.

Isolation of *Alternaria, Pythium, R. solani*, and other fungi from subcrown internodes also has been reported by others (1,4,14). The role of these fungi in the root rot complex is unclear. The association of AG-5 isolates of *R. solani* on wheat roots may be important in the epidemiology of the fungus on sugar beet. Cultures of AG-5 can reduce sugar beet seedling stands (25), and wheat often is rotated before sugar beet.

A significant correlation between percent subcrown internodes and crowns infected by *B. sorokiniana* or by *F. graminearum* and *F. culmorum* occurred, but not consistently. The high incidence of plants infected by these fungi in some fields likely reflects greater amounts of inoculum and/or favorable environmental conditions compared with fields where fewer roots were infected. Also, when inoculum is available and conditions are favorable, a high percentage of subcrown internodes and crowns could be infected, especially crowns, which have more surface area. Tinline et al (18) noted a direct relationship between intensity of subcrown internode lesions and reduction in fresh weight and grain yield, but not between intensity of external crown lesions and plant growth or yield.

The frequent recovery of *B. sorokiniana* from wheat subcrown internodes and crowns after anthesis compared with recovery of *Fusarium* species corresponds to results from other wheat-growing areas (1,4,18). Attempts to quantify conidia of *B. sorokiniana* (4,11) were unsuccessful because soil in northwestern Minnesota contains a high proportion of clay. Soil populations of *F. graminearum* and *F. culmorum* also were not determined because these fungi often are associated with organic matter. Duczek et al (6) demonstrated that as inoculum density of *B. sorokiniana* increases, disease incidence and severity increase. Wheat was the previous crop in all fields sampled, so populations of common root rot fungi would be expected to be high. Ledingham (9) found that when the duration of rotated crops not susceptible to common root rot increased, fewer wheat plants became infected. Fallow periods also resulted in a decrease in populations of *B. sorokiniana* and reduced root rot in wheat (4).

Table 1. Correlation coefficients (r)[a] for the relationship between recovery frequency of *Bipolaris sorokiniana* and *Fusarium graminearum* and *F. culmorum* isolated from subcrown internodes and crowns of spring wheat

	1985		1986		1987	
Variable set	**df**	***r***	**df**	***r***	**df**	***r***
Percent subcrown internodes vs. percent crowns with *B. sorokiniana*	10	0.48	17	0.57*	10	0.19
Percent subcrown internodes vs. percent crowns with *F. graminearum* and *F. culmorum*	10	...[b]	17	0.50*	10	0.79**

[a] * = $P = 0.05$, ** = $P = 0.01$.

[b] Infections of subcrown internodes too infrequent.

Cereal cultivars differ in susceptibility to common root rot (8,21). Marshall, the most widely grown cultivar in northwestern Minnesota, is less susceptible to common root rot than some spring wheat cultivars (C. E. Windels, *unpublished*) and also may account for the limited amount of root rot. Duczek et al (6) found that as inoculum density increased, susceptible wheat cultivars became more severely diseased than moderately resistant cultivars.

Of various management practices, including row spacings (1,21), seeding rates (1,17,21), seed size and seeding date (5), nitrogen fertilization (1,21), and tillage (2), planting depth is one factor that consistently influences the intensity of common root rot (1,5,17). For each year of this study, about one-third of the roots had no subcrown internodes, which indicates shallow planting. Because depth of planting varied within most fields, it is doubtful that shallow planting was intended to reduce root rot (21). A more probable explanation is that seed was drilled into soil compacted by tractor tires as the field was prepared for planting and during planting. Soil moisture also would affect depth of planting in compacted and noncompacted rows.

In conclusion, subcrown internode ratings of wheat were low, but *B. sorokiniana* frequently was isolated from subcrown internodes and crowns of roots in all disease classes, whereas *F. graminearum* and *F. culmorum* were isolated consistently, but at a lower frequency. *F. graminearum* group 2 was the predominant population of the species.

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