Late-Season Colonization and Survival of *Fusarium graminearum* Group II in Cornstalks in Minnesota

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ABSTRACT

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From 1973 through 1983, 14.6% of nearly 7,500 green cornstalks, collected from mid-September through early November, were infected by *Fusarium graminearum*. Of rotted (standing or lodged) stalks collected at the same time as green stalks in 1981 and 1982, 84.4% yielded *F. graminearum*. Recovery of *F. graminearum* from overwintered, standing cornfields the following spring was 61%, and recovery from 1-yr-old basal stalk pieces the following fall was 31%. Mature perithecia of *Gibberella zeae* developed in 99% of the isolates tested, which verifies the occurrence of the Group II population on corn in Minnesota. *F. graminearum* gains possession of pith tissue late in the growing season as stalks ripen and it predominates as tissues senesce, thereby increasing its inoculum potential and survival niches for the following season.

Additional key words: stalk rot

Throughout the world, *Fusarium graminearum* Schwabe and *F. moniliforme* Sheldon are the most frequently reported causes of Fusarium stalk rot on *Zea mays* L. (2,3,5,12,17). Early reports from the corn belt states of the United States and from other corn-growing regions named *F. graminearum* as the predominant cause of stalk rot (6,10). In recent decades, *F. moniliforme* has also been cited frequently as the major cause of cornstalk rot (5,22). Generally, basal or crown stalk rot of corn is attributed to *F. graminearum* and *F. moniliforme* (5), whereas nodal stalk rot is caused by *F. moniliforme* (22). Workers in Australia (9) further identified two populations of *F. graminearum* (Groups I and II). Group I isolates are mostly soilborne, cause crown rot of cereals, and rarely form perithecia. Group II isolates are mostly airborne, cause diseases of aerial plant parts (stalk and cob rot of corn, head blight of wheat, barley, and oats, and stub dieback of carnations), and normally form perithecia.

In preharvest surveys (September–October) made since 1973 (11,14,16) and in systematic biweekly sampling of cornstalks from anthesis to maturity in Minnesota (15), *F. moniliforme* was a frequently isolated parasite of symptomless cornstalks. We did not recover *F. graminearum* from cornstalks until September, when stalks were beginning to ripen (succulent pith tissue turning from green and firm-textured to straw-colored and pithy-textured) and usually did not exceed 20% colonization of stalks by early October. In these surveys, a bark increment hammer was used to remove samples from the basal portions of stalks (11,14–16). This proved to be a quick, easy, and nondestructive technique for sampling large numbers of plants. However, while sampling cornstalks in mid-October, when more than 90% of the dent corn ears are mature in Minnesota (but stalks are often still green), we occasionally encountered extensively rotted stalks and the bark increment hammer was ineffective in obtaining a sample. Isolations from these rotted stalks yielded *F. graminearum* in very high frequencies; this has been reported briefly (13).

The objectives of our study were to determine the frequency of *F. graminearum* as a late-season stalk invader of green and rotted stalks collected in mid-September through early November and to determine its ability to overwinter in standing stalks and in 1-yr-old stalk debris pieces.

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MATERIALS AND METHODS

Green stalks. From 1973 through 1983, 7,463 green (nonrotted) cornstalks of many cultivars were sampled from mid-September through early November in randomly selected fields in southern Minnesota, University of Minnesota Southern Experiment Station at Waseca, Southwest Experiment Station at Lamberton, and University of Minnesota, St. Paul. Of these samples, 359 stalks were collected on 1 and 22 October 1973, 300 on 1 October 1974, 300 on 28 October 1975, 867 on 8 and 15 September 1976, 1,000 on 12 September 1977, 985 on 12 October 1978, 1,500 on 17 and 26 October 1979, 1,630 on 17 and 24 October 1980, 111 on 22 October and 6 November 1981, 50 on 27 October 1982, and 360 on 26 September and 11 November 1983.

For studies made in 1973–1980, a bark increment hammer was used to remove a core (20–25 × 4 mm) from the first internode above the brace roots (11,14–16). The cores were surface-treated in 1% NaOCl for 30 sec, drained on paper towels, and placed on a medium selective for *Fusarium* species. During 1981–1983, stalk pieces were cut at the second or third internode above the brace roots, then severed at the soil line. These stalk pieces were stored for 24–48 hr at 5 C or outdoors. Stalks were slit open with a sterilized knife and a length (2–3 cm) of pith tissue was cut in the internode above the brace root with sterilized scissors, extracted with sterilized tweezers, and cultured on aureomycin-supplemented pentachloronitrobenzene (PCNB) agar, a medium selective for *Fusarium* species (19).

Rotted stalks. A total of 600 fallen and standing cornstalks that were rotted near the soil line (discolored externally and crushed when squeezed with fingers) were collected from several cultivars on 22 and 27 October 1981 and 27 November and 6 October 1982. Stalk pieces were stored and cultured as described for the green stalks.

Overwintered stalks. In April 1983, 100 overwintered cornstalks were collected from each of three randomly selected fields (one at Rosemount Experiment Station, University of Minnesota, and two at Olivia, MN) that were not harvested the previous fall. Basal stalk pieces were collected at random in each field because all plants crushed easily or were rotted. Collection of stalks and culturing procedures were followed as described previously.

Stalk debris pieces. One-year-old stalk debris pieces were collected from 18 cornfields in southern Minnesota that had been planted to corn the previous season (as evidenced by the presence of stalks on the soil surface in late September). Fifty basal cornstalk pieces were removed from the soil surface or

pulled from near the soil surface in each field, placed in paper bags, and stored outside for 1 wk until assayed. Because of advanced rotting of pith tissue, each stalk piece was reduced to the basal stem internode, about 0.5 × 1 cm, which still retained its integrity. The basal stem internode pieces were surface-treated in 1% NaOCl for 30 sec, drained on paper towels, and placed on PCNB agar (19).

Fusarium cultures were grown on PCNB agar under fluorescent lamps (12-hr photoperiod, 5,300 lux) for 1–2 wk, transferred to homemade and acidified potato-dextrose agar (PDA), and examined for *F. graminearum* 10–14 days later (18).

To test for the presence of Group I and II populations of *F. graminearum*, 109 isolates were hyphal-tipped onto carnation leaf agar (CLA) and incubated under fluorescent lamps (12-hr photoperiod) (18). After 1 mo, cultures were examined microscopically for mature ascospores in perithecia.

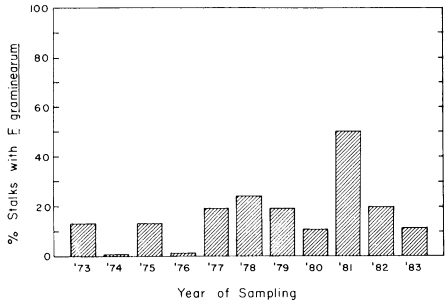

Fig. 1. Percentage of green (nonrotted) cornstalks collected from mid-September through early November from 1973 to 1983 that were colonized by *Fusarium graminearum*. Data for 1973–1977 were published previously: 1973 (14), 1974 and 1975 (15), 1976 (11), and 1977 (16).

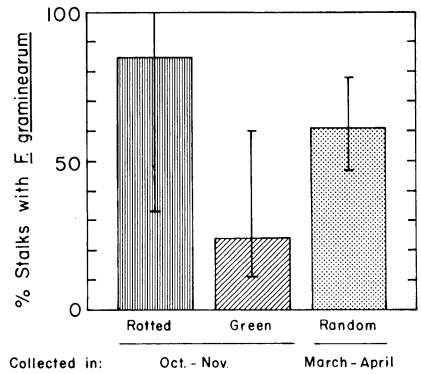

Fig. 2. Recovery of *Fusarium graminearum* from 633 rotted and 322 green cornstalks collected in October and November 1981–1983 and from 300 stalks of overwintering standing corn collected in March and April 1983. Lines within each bar show maximum and minimum recovery of *F. graminearum* from various cultivars and locations.

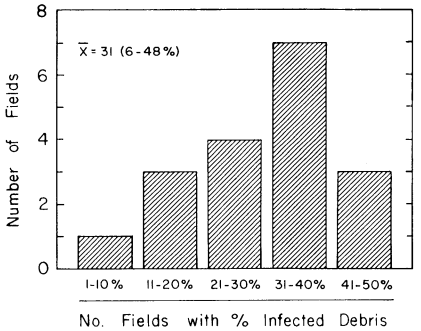

Fig. 3. Recovery of *Fusarium graminearum* from basal stalk internodes of 900 1-yr-old corn debris pieces; 50 samples were collected from each of 18 randomly selected "corn-on-corn" fields.

RESULTS

Green stalks. Of the 7,462 nonrotted cornstalks sampled from mid-September through early November over an 11-yr period, 14.6% were colonized by *F. graminearum*. As shown in Figure 1, recovery of the fungus ranged from 0.3 to 50.5%, but in 7 of 11 yr, it ranged from 10 to 20%. Green stalks collected at the same time as dead stalks in 1981–1983 yielded *F. graminearum* from 23.6% of the stalks. Although these stalks were firm when collected, many were in a transitional stage where pith tissue was turning from green with a crisp texture to straw-colored with a succulent to pithy texture. It is still possible to sample stalks in this condition with a bark increment hammer and they were thus obtained in the 1973–1980 surveys. In 1981 and 1982, nearly all stalks collected in late October and early November were ripened and dead, with a few in a transitional stage of ripening (therefore noted as green). However, in a field sampled in early November 1983, 80% of the stalks were green, although leaves had turned brown and ears had matured and had been harvested; *F. graminearum* was isolated from 6% of the green stalks and 33% of the rotted stalks.

Rotted stalks. Dead stalks were bleached in color externally and the pith tissue was dry to moist and ranged from straw-colored to pink-red. Sometimes, mycelial wefts were present in pith tissues, and brace roots and adventitious roots were also discolored (straw-colored, brown, pink, or red). Recovery of *F. graminearum* from dead stalks that had fallen or were still standing late in the season was 84.4%. Although not shown in Figure 2, *F. graminearum* was isolated from 89% (75–97%) of fallen, rotted stalks, and from 70.1 (33–100%) of the standing, rotted stalks. Often other *Fusarium* species grew from the stalk samples, as did bacteria and bacterial-feeding nematodes.

Overwintered stalks. *F. graminearum* was isolated from 61% of the corn stalks collected at random from three fields where cornstalks had not been chopped or plowed under the previous fall. Although not shown in Figure 2, *F. graminearum* was isolated from 47, 59, and 78% of the stalks in each of three fields. The field where 47% of the stalks were colonized was probably planted late because many of the cornstalks were light-colored but frozen when collected.

Stalk debris pieces. *F. graminearum* was recovered from an average of 31% (6–48%) of the basal stalk internodes of 1-yr-old cornstalk fragments collected from 18 randomly selected fields in southern Minnesota (Fig. 3). In more than half of the fields, *F. graminearum* was found in 36–48% of the basal stalk internode pieces.

More than 99% of the *F. graminearum* isolates from cornstalks that were hyphal-tipped produced perithecia of *Gibberella zeae* (Schw.) Petch bearing mature ascospores.

DISCUSSION

This study documents the occurrence of *F. graminearum* in cornstalks, but only late in the season, and reconfirms the conclusions of earlier workers that this fungus is the predominant stalk rot fungus in Minnesota (5,6,10). Recent studies in Minnesota and throughout the corn belt have shown the preponderance of *F. moniliforme* in cornstalks starting at anthesis and extending throughout the remainder of the growing season (12). Only by sampling rotted stalks late in the season was *F. graminearum* recovered with any frequency, although random sampling of green or "turning" stalks at the same time resulted in a regular but less frequent recovery of the fungus. About 70% of the 103 cultures isolated from these cornstalks and tested for pathogenicity resulted in stalk rot ratings where more than 50% of the inoculated internode was rotted (*unpublished*).

Throughout the growing season, the delicate balance between fungal activity within the stalk and the ability of the host to resist such activity varies with host genotype and the environment-host interaction and determines whether stalk rot develops in a plant or becomes prevalent in a field (5,7). By the end of the growing season, surveys and stalk samples can only monitor the resultant situation, and the variable nature of the disease becomes evident. For instance, in a previous survey of 50 randomly selected fields in southern Minnesota, the incidence of stalk rot (based on squeezing the basal internode) averaged 17% (0–82%) and incidence of lodged plants averaged 6% (0–45%) (14). In the last 10 yr, 90% (72–96%) of the dent corn grown in the state was rated mature during the first week of October and 55% (10–88%) was harvested by 24 October (Minnesota Weekly Crop-Weather Report, Minnesota Agricultural Statistics Service, St. Paul). However, mature grain does not necessarily mean that the stalks are showing overt signs of senescence or are beginning to decay. Over the decades, corn breeding programs have selected for prolonged longevity of pith tissue and

increased stalk strength (4,20), which undoubtedly have delayed invasion by stalk rot fungi from the infected roots. How much these breeding efforts, along with crop management practices that reduce plant stress, have delayed the onset of invasion by stalk rot organisms and/or stalk breakage can only be inferred at present from field observations.

The longer the harvest of corn is delayed, the more stalk tissues senesce and are susceptible to colonization or infection by *F. graminearum* and other opportunistic fungi. Premature ripening, hastened by early-season stress, can induce similar results. These conditions enable *F. graminearum* to build up its inoculum potential where it overwinters in debris on or near the soil surface and in standing corn as hyphae, chlamydospores, or perithecia (2). Survival in overwintered cornstalks and in basal stem fragments the following season does indicate that *F. graminearum* effectively retains possession of its substrate. In fact, association of *F. graminearum* with tissue appears essential to survival because the fungus is present in debris but is seldom isolated from soil collected in wheat (2) or corn fields (24). We have not determined whether the green stalk stubble left in fields after harvest is later colonized by *F. graminearum*.

The population of *F. graminearum* isolated in this study produces perithecia in culture, which is consistent with findings of Francis and Burgess (9) that Group II, or perithecium-forming populations, are associated with corn. Ascospores produced during the growing season can infect corn or nearby wheat fields and cause head scab (1,23). Infested corn residues incorporated into soil also serve as sources of infections as roots grow into this debris, and the stalk rot pathogen then grows into the stalks.

Young and Kucharek (25) suggested that communities of fungi function in succession in corn plants, although organisms may differ from season to season. Similarly, populations of *Fusarium* species in symptomless corn (15) and sorghum (21) plants change as the season progresses, with some species decreasing and others increasing in number. *F. moniliforme*, as a ubiquitous parasite of corn, apparently does not adversely affect mature corn (8), and damage may occur only under extraordinary circumstances. As plants mature, their resistance mechanisms decline and *F. graminearum* is more likely than *F. moniliforme* to compete for substrate and thereby overwhelm it. *F. moniliforme* and other fungal species may play a role in hastening the decay of stalk tissue and initiate the appropriate ecological sequence that ends with *F. graminearum* Group II as the climax species; however, this remains to be demonstrated.

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