Fungal Colonization of Stalks and Roots of Grain Sorghum During the Growing Season

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ABSTRACT

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Several fungal species were isolated from stalks and roots of grain sorghum (*Sorghum bicolor*) during the 1980 and 1981 growing seasons. Fungi were isolated with increasing frequency from anthesis through the grain-filling period. *Fusarium moniliforme* was the predominant species recovered from stalks. Several other *Fusarium* species were also found along with *Alternaria* spp. and *Nigrospora* sp. The fungi most commonly isolated from roots were *F. equiseti, Trichoderma* spp., and *Alternaria* spp. A similar sequence of colonization was observed in stalk tissue during the two seasons. Roots also appeared to be colonized by fungi in a pronounced sequence.

Stalk rots of sorghum (*Sorghum bicolor* (L.) Moench) are found worldwide wherever the crop is grown. With few exceptions, sorghum stalk rots are considered late-season diseases. They are characterized by degradation of pith tissue at or near the base of the stalk and are associated with the senescence of stalk pith cells that accompanies maturation and grain development. In the United States, stalk rots of sorghum are generally associated with three fungal species: *Fusarium moniliforme* Sheldon, *Macrophomina phaseolina* (Tassi) Goid., and *Colletotrichum graminicola* (Ces.) Wils. Several other species, however, have been reported to be isolated from diseased stalks at the end of the growing season (3). It is difficult to distinguish primary invaders from secondary organisms; therefore, the involvement of these fungi in the development of the disease is unclear.

Recent reports from studies of field corn (*Zea mays* L.) during the course of the growing season indicate that a diverse microflora exists within corn plants that display no apparent symptoms of stalk rot. Young and Kucharek (14) reported isolating 12 fungal species from corn stalk and root tissue during the growing season in Florida. These commonly isolated fungi were found to compose five distinct communities, which occurred in the maturing plants in a definite pattern of succession. In a study of *Fusarium* species associated with corn, Kommedahl et al (6) proposed that most, if not all, corn plants that have undergone anthesis are naturally colonized by species of *Fusarium*.

It is likely that a diverse fungal flora exists within stalks and roots of sorghum plants showing no symptoms of stalk rot in a manner similar to that of corn. In this study, we attempted to determine the validity of that hypothesis. With particular emphasis on *Fusarium* species, fungi were isolated from sorghum stalks and roots throughout the growing season. The purpose was to discover which species colonized stalk and root tissue and to determine if a pronounced sequence of infection could be observed.

MATERIALS AND METHODS

Field studies were made in 1980 and 1981 at the University of Nebraska North Platte Station. Unirrigated sorghum was grown as part of a long-term wheat (*Triticum aestivum* L.)-sorghum-fallow crop rotation system. Samples were taken from a different field each year, but both fields were located in the same vicinity and shared the same 19 yr of crop-rotation history. The plots from which plants were collected were sorghum-breeding test plots, and the samples consisted of several hybrid and inbred sorghum lines. All hybrids and lines were derived from the sorghum breeding program at North Platte and were similar in maturation rate. "No-tillage" cultivation was practiced on the test plots, so sorghum was planted directly into residue of the previous wheat crop.

Samples were collected at intervals throughout the growing season from 25 days after planting until after the first frost that was sufficiently severe to kill all top growth. Only plants showing no symptoms of stalk rot were sampled. Eight collections were made in both years, between mid-June and early November in 1980 and from early July to late October in 1981. Planting was delayed in 1981 because of cold wet conditions in May and early June. Rates of crop development in 1980 and 1981 were compared using Vanderlip's scale of sorghum developmental stages (12) and Nield and Seely's concept of growth-degree days (7) as well as our own observations (Table 1).

Stalk samples were collected at each sampling date, 200 in 1980 and 140 in 1981. Roots were examined in 1981 only. Random adventitious roots were removed from 70 root systems on each sampling date. The lower segment of each stalk specimen, consisting of the first six to eight nodes above the crown, was removed, cleaned, surface-sterilized for 3–5 min in 0.5% sodium hypochlorite, rinsed, and dried. Stalk segments were split longitudinally with a sterilized knife. Pieces of tissue (1 cm^3) were removed aseptically from the second, third, or fourth leaf node. A 4-cm segment of each root sample was washed, peeled to the cortical layer, and rinsed in distilled water. Stalk and root pieces were transferred to potato-dextrose agar (PDA) (11) supplemented with 10 mg/L of streptomycin sulfate.

After incubation at 25 C for 5–7 days, the plates were examined for fungal growth and colonies were identified to genus. Because our main emphasis was on the examination of *Fusarium* species, only a few isolates of each of the other genera were identified to species. These isolates were subcultured onto PDA and incubated at 25 C for 7 days. All *Fusarium* isolates were identified to species. To facilitate identification, they were subcultured onto a 2% water-agar medium containing pieces of sterile carnation leaves (11). Subcultures were

Table 1. Sorghum maturation rates in 1980 and 1981 at North Platte, NE, including planting and frost-kill dates

Sorghum growth stage	Approximate date	
	1980	1981
Planting	25 May	12 June
Flag leaf	15 July	3 Aug.
Boot	20 July	8 Aug.
Anthesis	28 July	21 Aug.
Soft dough	6 Aug.	4 Sept.
Hard dough	17 Aug.	21 Sept.
Physiological maturity	28 Aug.	NR[a]
Frost-kill	21 Oct.	14 Oct.

[a]NR = Not reached before the first killing frost.

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incubated at 20–25 C under continuous illumination from General Electric fluorescent "daylight" tubes (20W, 63 lm/W). *Fusarium* species were identified after 9–14 days. Although we adhered to the system of Wollenweber and Reinking (13) for standard nomenclature, we found it advantageous to combine for analysis those species placed in *F. roseum* Lk. emend. Snyd. & Hans. by Snyder and Hansen (10). With the exception of *F. graminearum* Schwabe and *F. equiseti* (Cda.) Sacc., which were treated as individual species, the following species were termed the "roseum" group and treated collectively: *F. scirpi* Lambotte & Fautr. var. *acuminatum* (Ell. & Ev.) Wr., *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., and *F. sambucinum* Fckl.

Fungal colonization of sorghum stalks and roots was measured with regard to individual species and to all species combined. A species was counted only once if it was isolated from a sample, even if several colonies developed. The number of samples that yielded a species was calculated as a percentage of total samples collected on that date and was termed "isolation frequency."

RESULTS

During 1980 and 1981, stalks and roots of sorghum were colonized by several fungal species. The mycoflora of roots was more diverse than that of stalks, but all species commonly found in stalks were also found in roots. The array of species isolated from stalks was similar in both years.

With maturation of the crop, the diversity of species isolated and the abundance of each species increased. By physiological maturity, as many as five species were often isolated from a single tissue sample. Fewer species were recovered from stalks in 1981 than in 1980, but multiple colonies of *F. moniliforme* frequently grew from the 1981 samples. The rate of fungal colonization of stalk tissue is best described as a sigmoidal pattern: a lag phase during the plants' vegetative growth stage, in which few fungi were isolated, was observed in stalks but not in roots (Fig. 1).

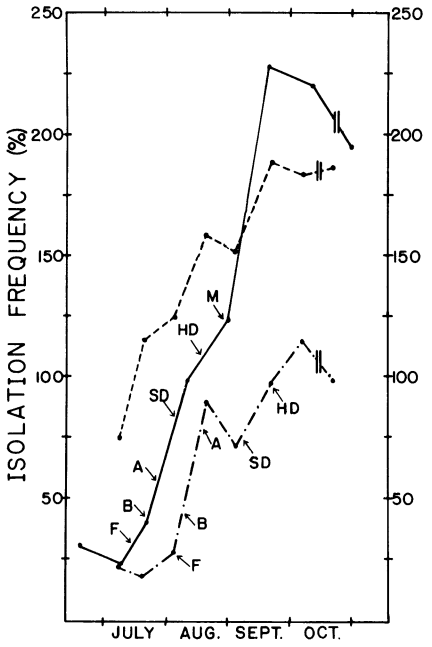

Fig. 1. Isolation frequency of fungal species in stalks (1980 and 1981) and roots (1981) of sorghum (stalks in 1980, •———•; stalks in 1981, •–·–·–•; and roots in 1981, •------•). Each point represents the number of species isolated expressed as a percentage of the total number of samples collected on that date (200 stalks in 1980, 140 stalks and 70 roots in 1981). Approximate dates of occurrence of plant developmental stages: flag leaf stage, F; boot stage, B; anthesis, A; soft dough, SD; hard dough, HD; and physiological maturity, M. || Represents the approximate date of the first killing frost.

Colonization of stalks. *F. moniliforme* was the dominant species isolated from stalks; it accounted for 25% of all species isolated in 1980 and 86% in 1981. *F. moniliforme* was recovered with a low frequency during vegetative growth of the host followed by an increase in isolation frequency during flowering and seed development (Fig. 2A). The isolation frequency decreased significantly during both years after the first killing frost.

In contrast to *F. moniliforme*, isolation frequencies of *F. graminearum* and the "roseum" group were low during the grain-filling period. These fungi were isolated from fewer than 25% of stalks sampled until after the first killing frost, when frequencies increased markedly (Fig. 2B,C). *F. tricinctum* (Cda.) Sacc. was isolated in a pattern similar to that of *F. graminearum* and the "roseum" group in 1980 (Fig. 2D). It was rarely recovered from stalks in 1981.

F. oxysporum Schlecht. emend. Snyd. & Hans. 'Redolens' and *F. solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. were recovered from as many as 20% of the stalk samples in 1980 but from fewer than 5% throughout 1981 (Fig. 2F). The isolation frequency of *F. equiseti* reached a maximum of 25% in 1980 and 12% in 1981 (Fig. 2E). The incidence of these three species differed from 1980 to 1981; their isolation frequencies increased at physiological maturity in 1980 but not in 1981.

Alternaria Nees spp. were recovered frequently from stalks during both seasons (Fig. 2G). Isolates that were identified to species were found to be *A. alternata* (Fr.) Keissler. In both years, the isolation frequencies of *Alternaria* spp. increased from the flag-leaf stage to anthesis. In 1980, the frequency continued to increase until after physiological maturity, when they colonized more than 50% of the stalks sampled. In 1981, the isolation frequency decreased after anthesis.

A species of *Nigrospora* Zimm. was commonly isolated from stalks in 1980 but rarely in 1981 (Fig. 2H). All isolates identified to species fit the description of *N. sphaerica* (Sacc.) Mason (4). The isolation frequency of *Nigrospora* sp. increased from the soft dough stage until 6 wk after physiological maturity in 1980.

Trichoderma Pers. ex Fr. spp. and *Epicoccum* Lk. ex Schlect. sp. were rarely recovered from stalk tissue in 1981 and never in 1980 (Fig. 2I,J). Selected isolates of *Trichoderma* spp. were identified as *T. viride* Pers. ex Gray (9). Only one *Epicoccum* species was recovered but its identity was not determined.

Although the relative abundance of each species differed from 1980 to 1981, a similar sequence of infection appeared to occur in stalks during both years. *F. moniliforme* and *Alternaria* spp. increased in frequency of recovery during the host's flowering period. *F. equiseti*, *F. oxysporum*, and *F. solani* did not increase in isolation frequency until later in the season if they increased at all. *F. graminearum*, *F. tricinctum*, and members of the "roseum" group were recovered with gradually increasing frequency throughout the season, with a marked increase after the aboveground portions of the plants were killed by frost. *Nigrospora* sp. appeared late when the plants reached physiological maturity.

Colonization of roots. *F. equiseti* was the *Fusarium* species most frequently isolated from roots (Fig. 2E). Combined, *F. oxysporum* and *F. solani* were almost as common as *F. equiseti* early in the season; however, the isolation frequency of these two species decreased to almost 0% by the end of the season (Fig. 2F). In contrast, the isolation frequency of *F. equiseti*, although erratic, remained high throughout the season.

F. moniliforme was rarely isolated from roots before the flag-leaf stage of plant development, but by anthesis, it was recovered from 30% of the samples (Fig. 2A). The isolation of *F. moniliforme* from roots was not adversely affected by the first frost; however, that frost was not severe enough to freeze the roots.

F. graminearum and members of the "roseum" group were recovered most frequently at the soft dough stage, when they were found in 25% of the roots sampled (Fig. 2B,C). *F. tricinctum* was consistently recovered from 5–10% of the samples (Fig. 2D).

Alternaria and *Trichoderma* spp. were common in root samples throughout the season (Fig. 2G,I). As in stalks, selected isolates of these two genera were identified as *A. alternata* and *T. viride*, respectively. The same *Nigrospora* sp. and *Epicoccum* sp. were found in roots as in stalks. *Nigrospora* sp. was rarely recovered from roots (Fig. 2H). *Epicoccum* sp. was recovered late in the season from about 40% of the samples (Fig. 2J).

A sequence of infection was observed in roots as well as in stalks. *F. oxysporum* and *F. solani* were isolated early, during the vegetative stage of plant development. *F. equiseti* and *Trichoderma* spp. were also isolated early, in a bimodal pattern. *F. moniliforme* and *Alternaria* spp. occurred with increasing frequency between the flag-leaf stage and anthesis. Isolation frequencies of *F. graminearum* and the "roseum" group increased gradually, reaching a maximum at the soft dough stage. *Epicoccum* sp. appeared just before physiological maturity.

DISCUSSION

Evidence from this study indicates that the general pattern of colonization of sorghum stalks and roots by fungi is much the same as in corn. The colonization of stalk tissue appears to depend upon the onset of flowering, but roots seem to be inhabited by fungi regardless of the growth stage of the plants. Fungi become more abundant in both stalks and roots as the crop matures. Similar observations have been made on corn (6).

The fungal species colonizing sorghum in western Nebraska are similar to those reported in corn, especially in the central United States. According to Christensen and Wilcoxson (2), *F. moniliforme* (Teleomorph: *Gibberella fujikuroi* (Sawada) Wollenw.) and *F. graminearum* (Teleomorph: *G. zeae* (Schw.) Petch) are commonly associated with ear and stalk rot of corn, and *Nigrospora* spp. are less common stalk rot pathogens of corn. *Trichoderma* and *Alternaria* spp. are frequently secondary invaders of rotted corn stalks. Of the *Fusarium* species we isolated from sorghum, all are reported to be associated with corn, usually as stem or root parasites (1).

The sequence of infection we observed in stalk tissue was similar in both years even though environmental conditions varied considerably from 1980 to 1981. From our observations, it appeared that the relative abundance of each fungal species was influenced by the difference in weather between the two seasons but that the sequence of infection was independent of those environmental changes. Perhaps it is more closely related to physical and metabolic changes within the stalk.

F. moniliforme was the dominant

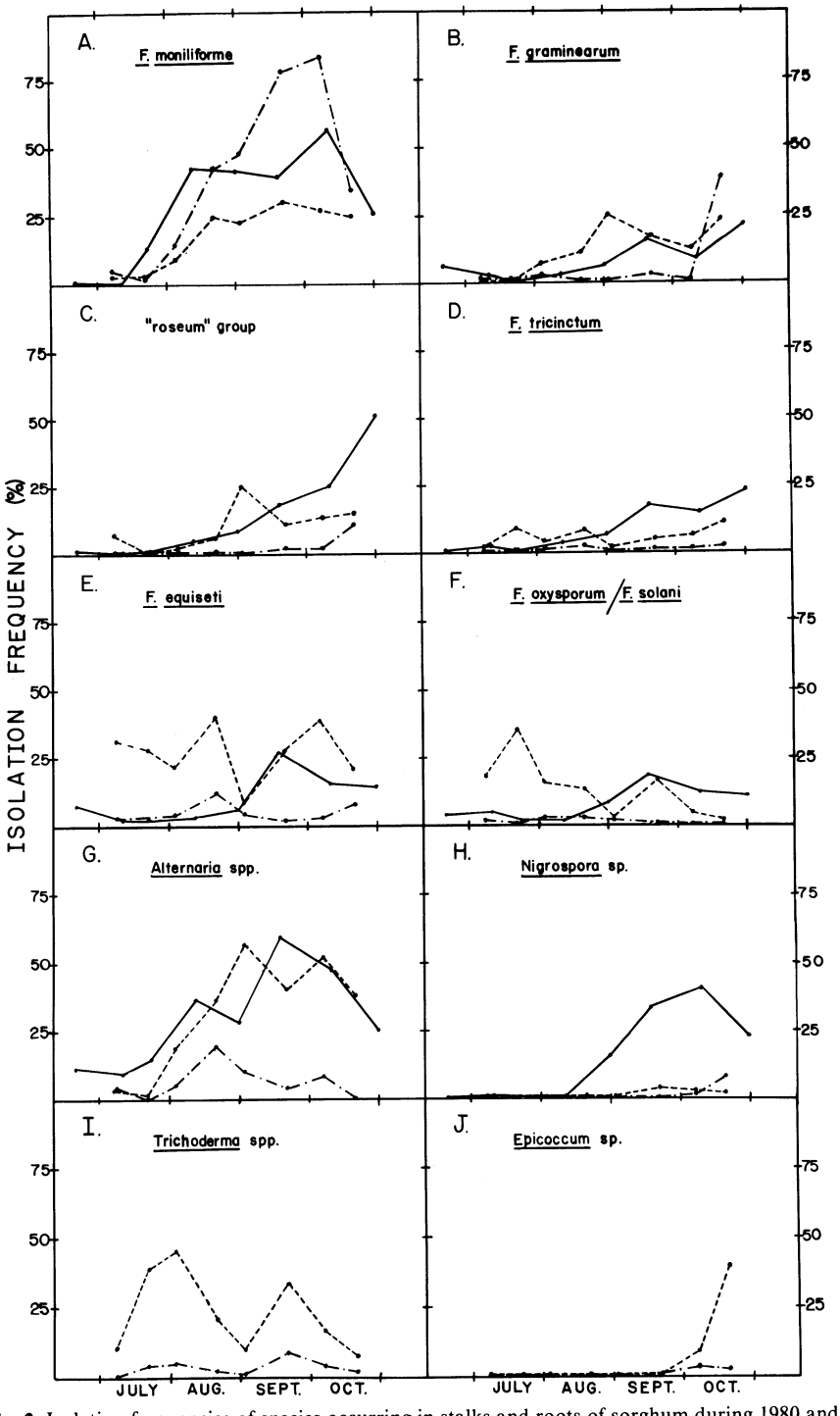

Fig. 2. Isolation frequencies of species occurring in stalks and roots of sorghum during 1980 and 1981. Species were treated individually with the following exceptions: 1) *Fusarium avenaceum, F. culmorum, F. sambucinum*, and *F. scirpi* var. *acuminatum* were considered collectively as the "roseum" group; 2) *F. oxysporum* (including *F. oxysporum* 'Redolens') and *F. solani* were treated together. Each point represents the frequency of occurrence of the species expressed as a percentage of the total number of samples collected on that date (200 stalks in 1980, 140 stalks and 70 roots in 1981). Stalks in 1980, •———•; stalks in 1981, •–·–·–•; roots in 1981, •-----•.

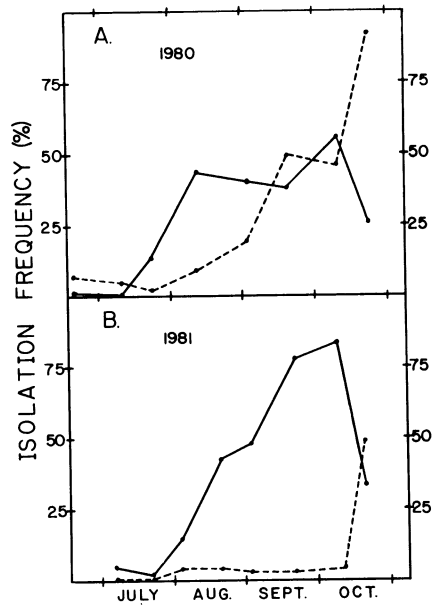

Fig. 3. Incidence of *Fusarium moniliforme* (•———•) compared with the combined incidence of *F. graminearum, F. tricinctum*, and the "roseum" group (•-----•) in stalks during 1980 and 1981. Each point represents the frequency of occurrence of the species (or group of species) expressed as a percentage of the total number of samples collected on that date (200 stalks in 1980, 140 stalks and 70 roots in 1981).

species in stalk tissue and apparently influenced populations of other *Fusarium* species. The trends in isolation frequencies of *F. graminearum, F. tricinctum*, and the "roseum" group were reciprocal to that of *F. moniliforme* during both years; when the incidence of *F. moniliforme* declined, the incidence of these other fungi increased, and the converse was also observed (Fig. 3). Populations of *F. equiseti, F. oxysporum*, and *F. solani* appeared to follow the same pattern in response to changes in the population of *F. moniliforme*. *F. moniliforme* appeared to be adversely affected by desiccation of stalk tissue as the plants approached maturity and by the first killing frost. Our observations indicate that when environmental conditions such as cooler temperatures and a greater moisture level in stalk tissue favor the growth of *F. moniliforme,* other species are less able competitors. These other species, however, appear to be better survivors and are able to colonize stalk tissue when the population of *F. moniliforme* is reduced.

The dominant fungi in sorghum roots were chlamydospore-forming *Fusarium* spp., *Alternaria* spp., and *Trichoderma* spp., which are all common root inhabitants. Because roots were only examined in 1981, we do not know if the sequence of infection we observed in roots will be repeated in subsequent seasons. The early colonization of roots may indicate that root infection leads to stalk infection. In some species, an increase in root populations appeared to precede an increase in stalk populations. However, because we did not observe whether root and stalk tissues of the same plant were colonized by the same species, we could not determine if a correlation actually existed. Root colonization is probably one of several ways by which stalk colonization occurs.

The association of stress conditions, senescence, and stalk rot of corn and sorghum are well documented (2,5,8), but the interrelationship between host and parasite(s) in the development of the disease is not understood. Evidence from this and other studies (6,14) demonstrates that fungi are present in stalk and root tissue in the absence of any symptoms of stalk rot. Colonization of stalks by fungi clearly does not, in and of itself, lead to the development of the disease. It is more likely that a balance exists between fungal activity within plant tissue and the ability of the host to withstand such activity. This balance may be shifted by factors adversely affecting the host or by conditions that favor increased fungal activity. The fungi may then become destructive to stalk tissue, leading to the development of stalk rot.

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