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Fungal Colonization of Alfalfa Stubble Following Harvest

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Two-year-old stands of alfalfa cultivars Saranac and Vernal, planted in Central Iowa, were sampled 0, 15, and 30 days after the first harvest in 1995 to identify fungi colonizing the stubble left after harvest and evaluate the role of harvest-induced wounds as infection sites for potential crown-rotting fungi. Analysis of variance was conducted to determine if samplig date and cultivar significantly affected incidence of the fungi. All stems were infected with at least one fungal species at every sampling date. The fungal genera most frequently isolated were *Alternaria, Colletotrichum, Fusarium, Phoma,* and *Rhizoctonia. Fusarium acuminatum* was the most frequently isolated *Fusarium* species. Other species included *F. sambucinum* and *F. sporotrichioides,* but not *F. oxysporum* or *F. solani.* Incidence of *Colletotrichum* decreased significantly over time, while the incidence of *Alternaria* increased significantly. Cultivar had a significant effect on *Colletotrichum* incidence, but not on incidence of other fungi. Potential crown rot pathogens were present on the stems at harvest, and their incidence generally did not increase significantly over time. These results do not provide evidence that wounds made by harvest equipment were important infection sites. Potential crown-rotting fungi were present in the stems at harvest; senescence of stem tissue after harvest may be important in the movement of fungi into the crowns.

Alfalfa (*Medicago sativa* L.) is the most important forage crop in the United States. In Iowa, it is grown on approximately 400,000 ha each year and is the third largest crop in area. Alfalfa is a perennial plant that usually is grown for three to four years in Iowa before replanting or rotating to another crop. As a perennial, alfalfa is subject to chronic disease problems. Plant density in most mature alfalfa stands declines over time due to disease-related mortality. When plant density drops below an economic threshold, the stand is terminated. Crown and root rots are the most frequently cited pathological causes of stand decline (Erwin 1954, Leath et al. 1971, Leath 1990a, Uddin and Knous 1991, Rodriguez and Leath 1992, Miller-Garvin and Viands 1994). Crown rot normally appears as a black or brown necrotic lesion that begins in the crown and extends into the tap root (Leath 1990a, Rodriguez and Leath 1992, Gossen 1994). Usually this rotting is in the cortical tissue of the crowns and roots, but also affects the vascular tissue (Leath et al. 1971, Leath 1990a). As the pathogens become established in the roots, the plant will decline and finally die due to the destruction of these nutrient absorbing and storing organs as well as destruction of crown buds. Crown and root rot is a complex that usually involves a combination of pathogens and other factors (Leath 1990a). The most common genera of pathogenic fungi isolated from rotted crowns include *Alternaria, Curvularia, Fusarium, Penicillium, Phoma, Pythium,* and *Rhizoctonia* (Erwin 1954, Hawn 1958, Leath 1990a, Uddin and Knous 1991, Gossen 1994).

The fungi colonizing alfalfa crown and root tissue may be either primary or secondary colonizers. Infection is believed to occur either through the root cortical tissue and into the crown by means of the xylem (Leath 1990a) or directly into the crown through a wound. Initial colonization often is followed by a succession of secondary colonizers.

Many factors in association with pathogens contribute to a decline in the stand density of alfalfa. Physiological stresses caused by weather events, such as winter injury or flooding, can cause the plants to be more susceptible to colonization. Harvesting practices also can influence susceptibility. Harvesting too frequently or at inappropriate growth stages will reduce plant vigor. Damage incurred to plants by harvesting equipment, insects, or livestock leave open pathways to crown disease (Gossen 1994, Undersander et al. 1994). In Iowa, hay is harvested three or four times a year. Harvesting causes a large number of wounds that are susceptible to fungal infection. Rodriguez and Leath (1992) showed that *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Boerema infected alfalfa stubble after cutting and proceeded into the crown to cause crown rot. After cutting, most stems die back to the highest remaining bud; some stems die back to the crown, providing a direct pathway for fungi to reach the crown. While some crown rots clearly can be traced back to cut stems (Munkvold, pers. obs.), the overall importance of stems as a pathway to crown rot has not been evaluated.

The objective of this research was to identify fungi colonizing alfalfa stems following harvest and to determine whether wounds caused by harvest equipment were major avenues for the fungal colonization of alfalfa crowns. This was accomplished by documenting temporal changes in the incidence of fungi isolated from stubble.

METHODS

The alfalfa sampled was located at the Iowa State University Agronomy Farm, eight kilometers west of Ames, Iowa. Two alfalfa cultivars, Vernal and Saranac, planted in 1993 in a replicated variety trial, were selected for sampling. Both cultivars are commonly planted in Iowa. Both have resistance to bacterial and Fusarium wilts, but lack resistance to anthracnose, Phytophthora root rot and Aphanomyces root rot (Brummer and Crim 1994). Vernal has a fall dormancy rating of two and Saranac, four. Three replicates of each cultivar were sampled on each of three dates following the first harvest in 1995. Plot areas sampled were 0.9 m by 3.7 m, containing approximately 180 plants each. Sampling was conducted immediately after cutting, 15 days after cutting, and 30 days after cutting. Forage was cut with a flail type cutter and removed from the plots immediately after cutting. Two live stems were sampled from each of five plants per replication; each plant was sampled only once during the study. Sampled plants, chosen arbitrarily, were labeled by attaching

a tag to the crown near the ground. Each stem sample consisted of approximately 2.5 cm of the distal end of live cut stems left on the crown after harvest. Stems were removed aseptically with scissors.

After stem samples were taken, a sterile scalpel was used to cut each stem piece in half longitudinally and then into 0.5 cm long segments. The pieces were surface sterilized in a 0.5% solution of sodium hypochlorite for two minutes, blotted dry with paper towels and then cultured on water agar and Difco potato dextrose agar (PDA); the PDA was amended with 200 mg/l of streptomycin sulfate, 50 mg/l of chlorotetracycline HCl, and 120 mg/l of neomycin sulfate, for isolation of fungi. The segments from one half of each bisected stem were placed on PDA and the segments from the other half of the stem were placed on water agar.

After two days at room temperature under fluorescent lights, the plates were inspected for growth. If possible, fungi were identified by fruiting structures and spores on the stem pieces. Individual colonies of unidentified fungi were then transferred to PDA to isolate pure cultures. From these cultures the fungi were identified to genus by microscopic examination of morphological characteristics. Incidence of each genus was recorded. *Fusarium* species were transferred to carnation leaf agar for species identification. Two-way analysis of variance was performed on the percentage of stems from which each fungus was isolated to evaluate the effects of cultivar and sampling date.

RESULTS

Measurable precipitation fell on 10 days during the sampling period, with a total amount of 11.2 cm. Precipitation did not occur on any of the sampling days. Mean temperatures during the sampling period were 26.7° C (mean daily maximum), 15.8° C (mean daily minimum), and 21.3° C (daily mean). On the three sampling dates, maximum and minimum temperatures were 25.5° C and 16.1° C (first sampling date), 31.7° C and 17.8° C (second sampling date), and 25.5° and 17.8° C (third sampling date).

All stems were colonized by several fungi at the time of harvest and at each subsequent sampling date. Approximately 950 individual fungal isolates were identified. The fungal genera found in the highest incidence included *Alternaria, Colletotrichum, Fusarium, Phoma,* and *Rhizoctonia* (all members of the Deuteromycotina). *Fusarium acuminatum* Ell. & Ev was the most frequently isolated *Fusarium* species; others included *F. sambucinum* Fuckel and *F. sporotrichioides* Sherb.

Morphology of *Colletotrichum* isolates varied substantially, such that they could be divided into two groups. In one group the colony color was tan to brown with abundant acervuli, setae, and conidia on PDA. In this group the conidial characteristics were consistent with *C. trifolii* Bain & Essary. In the other group, the colony color was gray to black with rudimentary acervuli but very few setae or conidia on PDA. These cultures produced abundant sterile fruiting structures. When transferred to sterile alfalfa stems, these isolates produced abundant setae and conidia. In this group the conidial characteristics were consistent with those of *C. destructivum* O'Gara.

The incidence of *Fusarium* spp. and *Rhizoctonia* did not change significantly over time or differ significantly between the two cultivars (Fig. 1, Table 1). The mean incidence of *Fusarium* spp. was consistent for both cultivars (94% for all sampling dates). The incidence of *Colletotrichum* decreased significantly over time for both Saranac and Vernal. However the incidence of *Colletotrichum* was significantly greater in Saranac, with a mean incidence of 83%, than in Vernal (55%). *Phoma* incidence did not differ significantly between the two cultivars or among sampling dates. The incidence of *Alternaria* was greatest at the second sampling date, 77% for Saranac and 90% for Vernal, and changed significantly over time; however the incidence was not significantly different between cultivars.

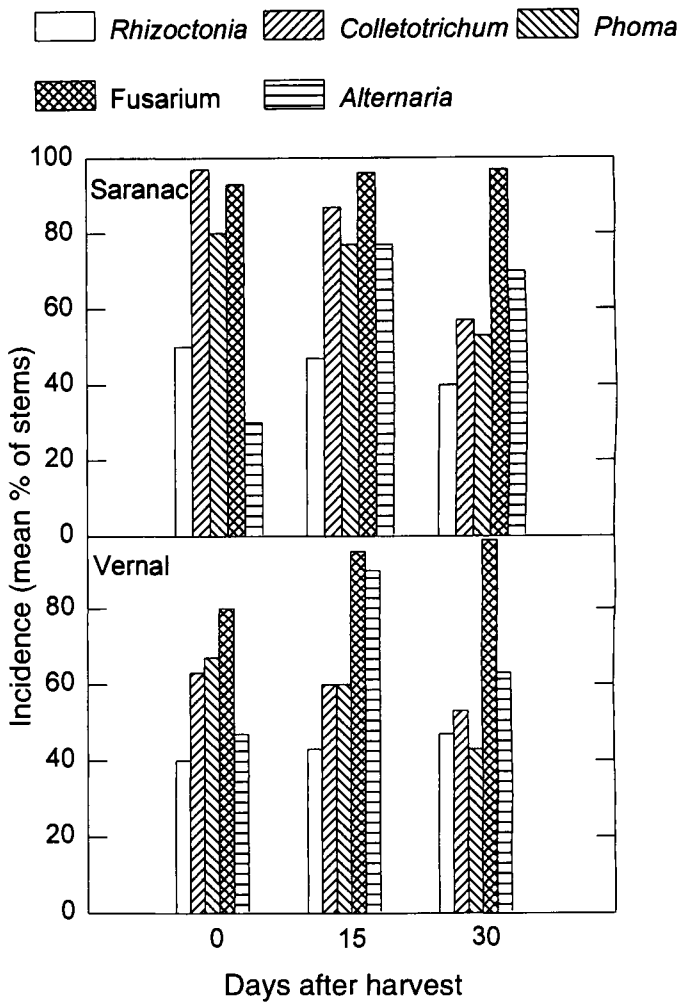

Fig. 1. Mean frequency of isolation of fungi from alfalfa stems after harvest. Each value represents the mean of three replications.

Table 1. Effects of cultivar and sampling date on incidence of fungi from alfalfa stubble, as determined by analysis of variance.

	Pr > F		
FUNGAL GENUS	CULTIVAR	SAMPLING DATE	CULTIVAR* SAMPLING DATE
Rhizoctonia	0.7961	0.9826	0.7234
Phoma	0.3067	0.3425	0.7543
Colletotrichum	0.0079	0.0114	0.6084
Fusarium	0.2781	0.2436	0.5266
Alternaria	0.3572	0.0034	0.4690

Other fungal genera isolated from the stems included: *Leptosphaerulina* and *Sordaria* (Ascomycotina), *Cladosporium* and *Trichoderma* (Deuteromycotina), and *Rhizopus* (Zygomycotina). Approximately 4% of the fungi isolated for each sampling date were not identified.

DISCUSSION

Association between crown rot and initial colonization through the stems has not been studied in much detail. The results of this study showed that several genera of fungi known to cause alfalfa crown rot can be isolated from stems at the time of harvest. As stems die back after harvest, these fungi may proceed into the crown and initiate crown rot. Because incidence of suspected crown rot pathogens did not increase significantly over time, we cannot conclude from these results that stem wounds made during harvest were important entry sites for these fungi. Incidence of *Fusarium* increased slightly over time, but the change was not significant. If crown rot pathogens are present in the stems prior to harvest, wounds may not be important as infection sites. However, the senescence of stem tissue after harvest may play a role in development of crown infections. This study was not designed to track movement of fungi toward the crowns. These issues need to be addressed directly through inoculation studies.

Differences between sampling times were not significant for most of the fungi colonizing the tissues. *Fusarium* and *Rhizoctonia* did not change much in incidence; most stems were infected by *Fusarium* at all sampling dates. *Alternaria* increased sharply, possibly due to the wounds made from the harvest equipment or due to senescence of tissue. The decrease in incidence of *Colletotrichum* may be due to competition by other fungi as the tissue senesced. Infection by *Phoma* is favored by cool weather (Leath 1990b); increasing temperatures during the course of the experiment may have been unfavorable for *Phoma* recovery at the later sampling dates. Fungi that typically occur later in the season, such as *Cercospora medicaginis* Ellis & Everh., were not recovered in this study, likely because of the time of sampling.

Fusarium species are among the most commonly cited causes of alfalfa root and crown rot, and the species involved include *F. acuminatum, F. avenaceum* (Fr.:Fr.) Sacc., *F. oxysporum* Schlecht, *F. sambucinum, F. solani* (Mart.) Sacc, and *F. sporotrichioides* (Leath et al. 1971, Leath 1990a, Uddin and Knous 1991, Gossen 1994, Miller-Garvin and Viands 1994). In this study, three of these species were isolated, but *F. oxysporum* and *F. solani* were notably absent. Although we have not shown that harvest wounds are important infection sites for any of the *Fusarium* species, the presence of *F. acuminatum, F. sambucinum,* and *F. sporotrichioides* in stubble suggests that these species may enter the crown through the stems, similar to *Phoma medicaginis* (Rodriguez and Leath 1992). Because *F. oxysporum* and *F. solani,* were not detected, we have no evidence that these two species utilize the stems as a pathway to the crowns.

At least three species of *Colletotrichum* are known to infect alfalfa, *C. destructivum, C. dematium* (Pers.) Grove f. *truncatum* (Schwein.) Arx., and *C. trifolii* (Ostazeski 1990). *C. trifolii* is believed to be the major cause of anthracnose in alfalfa; it can cause serious damage to stems and crowns, while the other two cause only minor damage (Ostazeski 1990). Although *Colletotrichum* spp. were commonly isolated, no anthracnose symptoms were observed. *Colletotrichum* spp. are known to infect many hosts without showing symptoms (latent infection). This latent colonization continues until symptoms appear due to plant stress (Sinclair 1991, Bailey et al. 1992, Prusky and Plumbley 1992).

The most common *Phoma* species isolated from alfalfa is *P. medicaginis,* the cause of spring black stem and leaf spot. This fungus can be isolated from leaves, stems and crowns (Leath 1990b, Rodriguez and Leath 1992), and is common in Iowa (Flynn et al. 1995). In the present study, *Phoma* isolates were not identified to species, but it is most likely that the isolates were *P. medicaginis.*

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