

# Soybean Pod Blight and Root Rot Caused by Lineages of the *Fusarium graminearum* and the Production of Mycotoxins

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## ABSTRACT

Surveys of soybean (*Glycine max*) seed grown in South Brazil revealed infection with *Fusarium graminearum*. To determine if members of this complex were pathogenic to soybean, six strains derived from soybean were added to soil at a rate of 10<sup>3</sup> macroconidia/ml or individual pods were inoculated with 10<sup>4</sup> macroconidia/ml. Seedlings grown in infested soil developed small necrotic lesions in the crown and upper roots. Pods inoculated with conidia developed large (>1 cm), dark brown, necrotic lesions. Younger pods inoculated with the fungus blighted and dropped from the plant. Strains of the *F. graminearum* complex recovered from lesions on the crown, roots and pods of soybean

plants were identified as lineage 1, 2 or 8 by obtaining the DNA sequence from the EF1-alpha gene and comparing it to strains of the known lineage. Two strains of *F. graminearum* lineage 7 from the U.S. caused similar symptoms of the disease on soybean. Mycotoxin tests on soybean and wheat (*Triticum aestivum*) indicate that most Brazilian strains produce nivalenol as the major trichothecene mycotoxin rather than deoxynivalenol. In addition, strains from lineages 2 and 8 produce the novel trichothecene, 3-acetylnivalenol.

**Additional keywords:** *Glycine max*, *Gibberella zeae*, pathogenicity, Deoxynivalenol, Nivalenol, compatibility groups.

## RESUMO

### Queima da vagem e podridão de raízes da soja causadas por linhagens de *Fusarium graminearum* e produção de micotoxinas

Amostras de sementes de soja (*Glycine max*) produzidas no Sul do Brasil estavam infetadas com *Fusarium graminearum*. Para determinar se membros desse complexo de espécies eram patogênicos à soja, seis isolados do fungo, originados de sementes soja, foram adicionados ao solo numa densidade de 10<sup>3</sup> macroconídios/ml ou vagens foram inoculadas individualmente com 10<sup>4</sup> macroconídios/ml. Plântulas crescidas em solo infestado desenvolveram pequenas lesões necróticas na coroa e na parte superior das raízes. Vagens inoculadas com conídios desenvolveram lesões necróticas marrom-escuro grandes (> 1 cm). Vagens jovens inoculadas com o fungo secaram e caíram da

planta. Isolados de *F. graminearum* recuperados das lesões das coroas, raízes e vagens das plantas de soja foram identificadas como pertencentes aos grupos de compatibilidade 1, 2 ou 8, através da análise da sequência de DNA do gene EF1-alfa, quando comparados com as linhagens conhecidas. Dois isolados de *F. graminearum* dos Estados Unidos, pertencentes ao grupo de compatibilidade 7, causaram sintomas semelhantes em soja. Testes das micotoxinas produzidas em soja e trigo (*Triticum aestivum*) indicaram que a maioria dos isolados Brasileiros produz Nivalenol, como a principal toxina dos tricotecenos, ao invés de Deoxinivalenol. Em adição, os isolados dos grupos 2 e 8 produziram uma nova toxina, o 3,Acetil-Nivalenol.

## INTRODUCTION

Members of the *Fusarium graminearum* Schwabe complex (hereafter referred to as the *Fg* complex) are as pathogens of cereal crops found throughout the world. During the 1990's, members of this complex were largely responsible for the highly destructive *Fusarium* head blight (scab) epidemics of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in North and South America, Asia and Europe (Dubin *et al.*, 1997). The re-emergence of *Fusarium* head blight as an important constraint to small grain production may be attributable, at least in part, to the increase of low- or no-till cropping systems worldwide. These cultural practices leave crop residue on the soil surface where it may be colonized by

saprophytic or potentially pathogenic fungi. *Fusarium graminearum* has been shown to readily colonize crop debris left behind by wheat, corn (*Zea mays* L.) and other rotation crops (Cook, 1984; Wicklow *et al.*, 1987; Fernandez, 1991; Miller *et al.*, 1998).

Soybean [*Glycine max* (L.) Merrill] is often used in rotation with wheat and other cereal crops. Soybean crop residues in fields under conservation tillage have been found to be heavily colonized by *F. graminearum* (Wicklow *et al.*, 1987; Fernandez & Fernandes, 1990; Baird *et al.*, 1997). Although members of this species complex also reportedly colonize living soybean stems (Harrington *et al.*, 2000) and seeds (Osorio & McGee, 1992; Jacobsen *et al.*, 1995), many authors consider members of the *F. graminearum* complex to

be nonpathogenic to soybean (Chamberlain, 1972; Fernandez & Fernandes, 1990; Garcia-Romera *et al.*, 1998; Miller *et al.*, 1998).

Published reports of disease on soybean caused by the *F. graminearum* complex are equivocal, circumstantial and/or contradictory. Anderson *et al.* (1988) reported isolation of these fungi from diseased and stunted soybean in Ontario, Canada at a fairly high frequency (18%). However, they were also isolated at a similar rate (14%) from plants showing no symptoms. *Fusarium graminearum* has been considered a secondary colonist of soybean seed damaged by other fungi or by freezing (Osorio & McGee, 1992; Jacobsen *et al.*, 1995; Ward *et al.*, 2002). Although Agarwal (1976) reported *Fusarium* root rot of soybean caused by *F. graminearum* in India, the description of the pathogen, especially the lack of a homothallic sexual stage, may implicate the fungus now known as *F. pseudograminearum* O'Donnell & T.Aoki (Aoki & O'Donnell, 1999), [formerly known as *F. graminearum* group 1 (Francis & Burgess, 1977)] as the actual pathogen in that report. Other reports appear to suggest that the *F. graminearum* complex (teleomorph = *Gibberella zeae*), is nonpathogenic to soybean even at high inoculum levels (Garcia-Romera *et al.*, 1998), or that it may even protect the plant against other root diseases (Chamberlain, 1972).

Soybean production in South Brazil involves no-till cultivation and double cropping rotation with wheat, barley or oats (*Avena sativa* L.). Large inoculum levels of *F. graminearum* are present under these conditions and we have observed that members of the *F. graminearum* complex appear to be affecting plants previously considered nonhosts. Oats, for example, now are susceptible to scab disease and perithecia may even form on maturing panicles in the field (J. Martinelli, personal observation). Recent surveys of soybean seed grown in South Brazil revealed infection by members of the Fg complex. Seed lots varied in the percentage of infected seed, ranging from 0 - ca. 20%. Therefore, the objective of this study was to determine whether strains of the *F. graminearum* complex obtained from soybean seeds are pathogenic to soybean and to determine their genetic lineage as well as their ability to produce toxins. An abstract of this work was published previously (Martinelli *et al.*, 2001).

## MATERIALS AND METHODS

### Origin and identification of the isolates

Brazilian soybean seeds were surface-disinfested with 1.0% sodium hypochlorite for 2 min and plated on 2% water agar or one-quarter strength potato dextrose agar (PDA - Difco; Detroit, MI). After eight days, conidia were examined from six colonies growing independently from the seeds and determined by morphology (Aoki & O'Donnell, 1999) to be members of the *F. graminearum* complex. Monosporic cultures were grown on mung bean agar (Evans *et al.*, 2000) to obtain inoculum for subsequent pathogenicity experiments. For comparison of pathogenicity, three additional strains were used: one isolated from wheat in Brazil, one from wheat (NRRL 29169) and corn (NRRL 31084) in the US.

To determine the lineage of the Brazilian *F. graminearum* complex (Table 1) isolates obtained from soybean and wheat, a portion of the gene encoding translation elongation factor EF1-alpha was amplified and the DNA sequences were compared to those previously published from strains of known lineage (O'Donnell *et al.*, 2000). In that work, allelic genealogies were constructed from DNA sequence of six single-copy nuclear genes from 27 strains of *F. graminearum* selected to represent the global genetic diversity of this pathogen. With the exception of one strain, all six genealogies recovered the same seven biogeographically structured lineages suggesting they represent phylogenetically distinct species among which gene flow has been very limited historically.

### Test with seedlings and adult plants

To determine if strains of the *F. graminearum* complex are capable of causing disease symptoms on soybean, macroconidia of the six strains isolated from soybean were used to challenge seedlings and pods on adult plants of the American soybean varieties Glacier, Parker and Lambert.

A macroconidial suspension (10 ml at  $10^3$  spores/ml) was added to pots (5x5x5 cm) with vermiculite containing 11-day-old plants or to pots (13 cm height, 8.5 cm diameter at base and 12.5 cm diameter on top) with soil (pasteurized mix of field soil, sand, peat moss, composted manure 6:6:5:2) immediately prior to sowing seeds. Roots of seedlings in vermiculite pots

TABLE 1 - Strains of *Fusarium graminearum* used in this study

NRRL # / CDL # <sup>1</sup>	Host	Cultivar	Country	State <sup>2</sup>	Lineage
31230 = 01-105	Soybean	Bragg	Brazil	RS	2
31321 = 01-156	Soybean	BRS66	Brazil	RS	8
31322 = 01-157	Soybean	BRS66	Brazil	RS	2
31323 = 01-158	Soybean	BRS66	Brazil	RS	1
31351 = 01-186	Wheat	BRS192	Brazil	Paraná	8
31354 = 01-189	Soybean	FT Abyara	Brazil	Paraná	2
31355 = 01-190	Soybean	FT Abyara	Brazil	Paraná	8
29169	Wheat	Unknown	USA	Kansas	7
31084	Corn	Unknown	USA	Michigan	7

<sup>1</sup> Numbers given to strains by culture collections at the National Center for Agricultural Utilization Research (NRRL) or the Cereal Disease Laboratory (CDL).

<sup>2</sup> RS = Rio Grande do Sul.

were wounded with a knife just before inoculation whereas seedlings grown in soil were not wounded. For the latter, five holes per pot were made with a pencil by pushing it from the soil surface to the bottom of the pot after which the spore suspension (10 ml at  $10^3$  spores/ml) was added into each hole. One seed was then placed on top of each hole and they were then covered with a 3 cm layer of soil. Each isolate was added to four pots per variety containing either four (vermiculite) or five (soil) seedlings. Each soybean variety was tested for both seedling experiments, and for both experiments bedding material treated with water was used as a negative control. After addition of inoculum, seedlings were grown for 4 weeks after which the roots and crowns were examined.

To test for the ability of the strains to cause disease on developing soybean seeds and pods, seeds of the same three varieties were planted in larger pots (15.5 cm height with a 10 cm diameter at the base and a 15 cm diameter at the top), one plant per pot, five pots per treatment and grown to the adult stage. At the beginning of seed formation (R5 stage), pods were inoculated with the strains by injecting 0.1 ml of a spore suspension containing ca.  $10^3$  conidia/ml. To determine if the fungus could invade beyond the point of inoculation in most cases the pods were inoculated on the mid-lateral surface by injecting spores into a central carpal containing a developing seed. Plants inoculated with sterile water were included as a negative control.

#### Recovery of isolates

Seedlings were removed from pots four weeks after inoculation to test for fungal growth from the roots. Roots were excised, washed, surface-disinfested and plated on one-quarter strength PDA. A similar procedure for identifying colonization of above ground tissue was used for inoculated pods and seed harvested from adult plants.

#### Testing soybean isolates on wheat

All Brazilian strains also were inoculated on wheat heads at anthesis to determine if the isolates from soybeans could cause disease on wheat. A drop (ca. 0.05 ml) of spore suspension from each isolate containing  $10^3$  spores/ml was inoculated into either a central spikelet of one head or on four other heads. The same spore suspensions were sprayed over the entire surface of the inflorescence until runoff. After inoculation the plants were kept in a dew chamber for 72 h and then transferred to the greenhouse to be evaluated ten days later for symptoms of Fusarium head blight.

#### Gas chromatography/mass spectrometry (GC/MS) analysis of toxins

Samples of pods showing symptoms of disease caused by *Fusarium* spp. seven days after inoculation were assayed as were soybean seeds and pods, and wheat heads ten days after inoculation. Each was analyzed for trichothecene toxins. Since the inoculated wheat did not produce any seed, entire heads were harvested. All samples were ground to a fine powder for toxin analysis according to the method described by

Mirocha *et al.* (1998) with modifications. Briefly, 1 g of wheat heads, 2 g of soybean pods or 3 g of soybean seeds were extracted with 10 ml, 16 ml and 16 ml of acetonitrile:water (84:16 v/v), respectively, in 50-ml centrifuge tubes for 1 h. An aliquot of 1 ml of the extract after column cleanup was placed into a 1/2 dram vial and evaporated to dryness under nitrogen. The sample was derivatized with 20  $\mu$ l of trimethylsilyl ether (TMS) reagent (TMSI/TMCS 100:1, Sigma) and diluted with 200  $\mu$ l of iso-octane. The GC/MS sample analysis was carried out on a Shimadzu QP5000 Gas chromatography/mass spectrometry system with a Shimadzu AOC-1400 autosampler and AOC-17 autoinjector (Shimadzu, Kyoto, Japan). Quantitative analysis was run in selected ion monitoring (SIM) mode. Ions at m/z 235.10, 259.10, 295.10, 377.10 and 392.15 were monitored. A full scan GC/MS analysis (scan range m/z 50 to m/z 600) was carried out for toxin identification of the wheat head samples. The GC column was a J&W DB-5ms, 0.25  $\mu$ m film thickness, 250  $\mu$ m i.d. and 30  $\mu$ m in length. The GC temperature program included an initial temperature of 150 °C for 1 min, a ramp to 280 °C at 25 °C/min, and an isothermal for 5 min.

## RESULTS

#### Lineage of strains derived from soybean

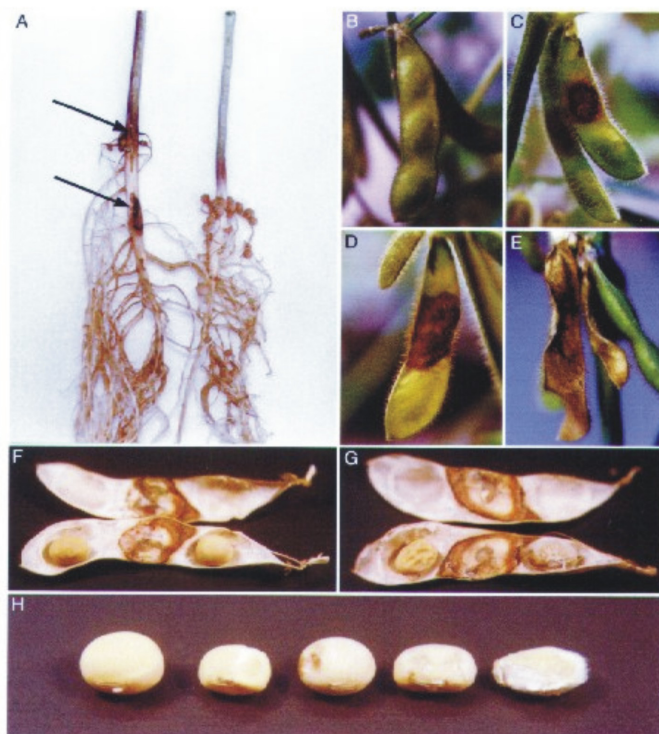
The alleles from the Brazilian strains correspond either to those of strains previously described as lineage 1 or 2, or from a newly reported group called lineage 8 (Table 1). Strains of lineage 8 also have been found in New Zealand.

#### Symptoms and recovery of the fungus from diseased tissue

When seedlings were grown in soil or vermiculite infested with different strains of the *F. graminearum* complex, symptoms of disease appeared on plants from all treatments whereas the negative controls remained symptomless. While most of the plants in each treatment also were free of symptoms, at least one plant per pot exhibited macroscopic necrotic lesions at the soil line in the crown and upper roots. Lesions were distinct, longitudinally elongate, and varied in color from brown to black (Figure 1). These symptoms are similar to those described for Fusarium root rot of soybean caused by members of the *F. solani* (Mart.) Sacc. and *F. oxysporum* Schltdl species complexes (Hartman *et al.*, 1999). To determine if fungi other than the *F. graminearum* complex could be causing the same symptoms in treatments and to complete Koch's postulates, symptomatic root tissue was plated on 1/4 strength PDA agar. Fungal colonies arising from all lesions were determined by morphology to be members of the *F. graminearum* complex. Members of this complex were not recovered from roots from plants grown in non-infested soil.

Because strains of the *F. graminearum* complex tested were obtained from soybean seed, adult plants also were inoculated to determine if the strains could cause disease on inoculated pods and seed. All strains tested, including isolates originally obtained from wheat and corn, produced disease on inoculated soybean pods (Figure 1). All strains caused the development of circular, chocolate brown lesions at the point





**FIG. 1** - Symptoms of disease caused by strains of the *Fusarium graminearum* complex on soybean (*Glycine max*). A) (left) lesions on crown and hypocotyl of soybean (arrows) grown in soil infested with *F. graminearum* and, (right) non-symptomatic soybean grown in noninfested soil, B) mock-inoculated control for soybean pod assay C) inoculated pod showing discrete, chocolate-brown lesions, D) a dry, tan to brown, expanding pod lesion, E) blighting of young inoculated pods, F) internal symptoms of pod showing necrosis limited to the central inoculated carpal, G) internal symptoms of pod showing spread of infection to adjacent seed, and H) seed from inoculated pods ranging from non-symptomatic (left), to a brown necrotic spot (center) or largely colonized by fungal hyphae (right).

of inoculation. Lesions tended to spread more in younger pods, causing them to yellow, wither and blight within seven days of inoculation. Seeds failed to develop in the carpals of those pods that were inoculated with spores by injection. Seeds that formed in pods distal to the point of inoculation were harvested and plated on ¼ strength PDA agar to confirm infection by *F. graminearum*.

#### Virulence of soybean isolates on wheat

To determine whether strains of the *F. graminearum* complex from soybean could cause Fusarium head blight disease on wheat, six strains obtained from soybean (NRRL 31230, NRRL 31321, NRRL 31322, NRRL 31323, NRRL 31354 and NRRL 31335) were inoculated on wheat heads and compared in their reaction to a strain of lineage 7 known to cause wheat head blight (NRRL 29169). Without exception, all of the soybean isolates were highly virulent on wheat, similar to the NRRL 29169, particularly when the spore suspensions were sprayed on the heads. Spray inoculation of heads resulted

in profuse mycelial growth that covered the inflorescence after incubation in the moist chamber. Blighting occurred to each head to the extent that no seeds developed.

#### Toxin production

Seven days after inoculation, the central necrotic portion of soybean pod tissue was excised, freeze-dried and analyzed for deoxynivalenol (DON) content. Among the soybean strains only NRRL 31323 (lineage 1) and NRRL 31354 (lineage 2) produced detectable levels of DON after seven days, accumulating to 0.6 and 2.0 ppm, respectively. Both U.S. strains from nonsoybean hosts (NRRL 29169 and NRRL 31084) also produced DON but in larger amounts, 6.2 and 5.8 ppm, respectively.

In a separate experiment, to test if soybean strains were capable of producing mycotoxins at later stages of disease, toxins were analyzed at plant maturity on harvested seeds and pods. Strains from soybean generally produced more nivalenol than DON, with the exception of NRRL 31323 (lineage 1) which was a DON producer (Table 2). While inoculated at the same time and treated under identical conditions, soybean variety Parker appeared to accumulate negligible amounts of mycotoxins compared to Glacier and Lambert. As in the previous experiment, the U.S. isolates (Fg complex lineage 7) produced larger amounts of mycotoxin in the form of DON and no detectable nivalenol.

To test if soybean strains were capable of producing mycotoxin when inoculated on wheat heads, diseased tissue was analyzed for trichothecenes two weeks after inoculation. In all of the isolates tested, a higher level of mycotoxin accumulation was observed in wheat than in soybean seed or pod tissue (Table 3). As suggested by the results on soybean, all Brazilian strains except NRRL 31323 produced mainly nivalenol. One soybean isolate from Brazil (NRRL 31323) and the wheat isolate from the US (NRRL 29169) produced mainly DON. Brazilian lineage 2 and 8 nivalenol producers also were capable of producing a novel trichothecene derivative identified as 3-acetylnivalenol (Table 3). This toxin has been recently reported in a separate publication confirming its identity and structure (Rodrigues Filho *et al.*, 2002).

#### DISCUSSION

Strains of the *F. graminearum* complex were readily isolated from soybean seed lots obtained from Brazil. Soybean seed, roots, and plant debris all have been reported to harbor members of this complex (Ward *et al.*, 1987; Fernandez & Fernandes, 1990; Harrington *et al.*, 2000; Baird *et al.*, 1997) and to cause seed discoloration as a secondary colonist (Osorio & McGee, 1992; Jacobsen *et al.*, 1995; Ward *et al.*, 2002). However, to our knowledge, this is the first report to unambiguously demonstrate pathogenicity of members of the *F. graminearum* complex to soybean as a primary pathogen by applying Koch's postulates. Members of this complex consistently caused disease on all soybean varieties under all conditions tested.

An earlier report of root rot of soybean caused by *F.*

**TABLE 2** - Production of deoxynivalenol (DON) and nivalenol (NIV) toxins in soybean (*Glycine max*) seeds or pods by members of the *Fusarium graminearum* complex

Variety / Isolate	Amount of toxins (parts per million)			
	In seeds		In pods	
	DON	NIV	DON	NIV
1) Glacier <b>Negative Control</b>	nd	nd	nd	nd
2) Glacier / 31230	nd	0.2	0.01	0.7
3) Glacier / 31321	nd	nd	nd	nd
4) Glacier / 31322	0.1	0.4	nd	nd
5) Glacier / 31323	2.4	nd	2.8	nd
6) Glacier / 31351	nd	nd	nd	nd
7) Glacier / 31354	0.04	1.3	nd	3.8
8) Glacier / 31355	0.03	1.5	nd	3.1
9) Glacier / 29169	13.5	nd	28.8	nd
10) Glacier / 31084	7.0	nd	8.8	nd
11) Parker <b>Negative Control</b>	nd	nd	nd	nd
12) Parker / 31230	nd	nd	nd	nd
13) Parker / 31321	nd	nd	nd	nd
14) Parker / 31322	nd	nd	nd	nd
15) Parker / 31323	nd	nd	nd	nd
16) Parker / 31351	nd	nd	nd	nd
17) Parker / 31354	nd	nd	nd	nd
18) Parker / 31355	nd	nd	nd	nd
19) Parker / 29169	nd	nd	nd	nd
20) Parker / 31084	0.2	nd	nd	nd
21) Lambert <b>Negative Control</b>	nd	nd	nd	nd
22) Lambert / 31230	nd	nd	nd	nd
23) Lambert / 31321	0.01	0.5	nd	nd
24) Lambert / 31322	0.06	0.4	0.7	nd
25) Lambert / 31323	0.2	nd	nd	nd
26) Lambert / 31351	nd	0.4	nd	nd
27) Lambert / 31354	0.4	23.4	0.1	9.6
28) Lambert / 31355	0.02	0.6	nd	nd
29) Lambert / 29169	51.5	nd	51.5	nd
30) Lambert / 31084	5.1	nd	11.3	nd

*graminearum* (Agarwal, 1976) must be considered ambiguous since no mention is made in this publication of a *Gibberella zeae* (Schwein.) Petch sexual state. Lack of a description of a sexual stage suggests that the causal agent may have been the heterothallic fungus now known as *F. pseudograminearum* (*G. coronicola* T.Aoki & O'Donnell) (Aoki & O'Donnell, 1999)

**TABLE 3** - Toxins produced by members of the *Fusarium graminearum* complex grown in wheat (*Triticum aestivum*) heads

NRRL # / lineage	Amount of toxins (parts per million)		
	deoxynivalenol	Nivalenol	3-acetylivalenol <sup>a</sup>
31230 / 2	0.2	46.7	+
31321 / 8	1.0	131.1	+
31322 / 2	5.8	59.5	+
31323 / 1	731.1	nd	nd
31351 / 8	0.9	247.2	+
31354 / 2	1.7	207.3	+
31355 / 8	0.4	65.3	+
29169 = 7	520.1	nd	nd

<sup>a</sup> = Indicates 3-acetylivalenol was detected. This compound was not quantified because it is unavailable commercially for use as a concentration standard. nd = not detected.

and not the homothallic fungus, *F. graminearum*, which readily forms *G. zeae* perithecia in culture. Attempts to locate a culture of the fungus described by Agarwal (1976) were unsuccessful.

Because the *F. graminearum* complex lineages we examined caused root and crown rot disease at a relatively low concentration of spores, we conclude that these fungi must be considered legitimate contributors to the Fusarium root rot complex of soybean in Brazil, since the inoculum of this pathogen in soybean debris may reach levels ten times greater than that tested here (Fernandez & Fernandes, 1990). Currently, members of the *F. oxysporum* and *F. solani* species complexes are recognized as pathogens within this disease complex (Hartman *et al.*, 1999). Given that representatives of *F. graminearum* complex lineage 7 from the U.S. also caused root and crown rot disease in the greenhouse, and that levels of the pathogen in soybean debris in the U.S. are also very high (Baird *et al.*, 1997), we suggest that greater attention be given to the potential contribution of the *F. graminearum* complex to the soybean root rot problem in the U.S.

Members of the *F. graminearum* complex also were reported to infect soybean seeds causing a reddish discoloration of the seed coat concomitant with the accumulation of deoxynivalenol (Wicklow *et al.*, 1987; Jacobsen *et al.*, 1995; Rodrigues Filho *et al.*, 2002). Wicklow *et al.* (1987) reported that *Fusarium* infection occurred only in damaged seed previously infected with *Peronospora manshurica* (Naumov) Syd. Likewise, Osorio & McGee (1992) concluded that while soybean seed damaged by freezing injury was reduced in viability by *F. graminearum* complex colonization, these fungi had little effect on healthy seed. Finally, Jacobsen *et al.* (1995) noted similar colonization of seed coats during a year when the crop was damaged by allowing the mature seed to remain in the field for an extended period of time, resulting in the colonization of senescing pod tissue by a variety of opportunistic saprophytic fungi including *F. graminearum*.

The disease caused by several lineages of the *F. graminearum* complex on soybean pods reported here is much different from the symptoms described for colonized seed coats reported previously. Our inoculations resulted in spreading necrotic lesions on healthy pod tissue weeks before onset of naturally occurring senescence. Successful inoculations caused pod blighting and the infection sometimes expanded within the pod to completely colonize the seed coat, endosperm, and embryo of adjacent developing seeds. However, the reddish-pink discoloration of the soybean seed coat previously reported for *F. graminearum* was not observed. Instead, the *F. graminearum* infection described here included formation of brown, sunken necrotic spots on seeds as well as signs of the fungus as fluffy white mycelium arising from heavily infected seed. Extensively colonized seed was not viable. Taken together, the symptoms reported here more closely resemble pod and collar rot of soybean caused by *F. pallidoroseum* (Cooke) Sacc. (Hartman *et al.*, 1999) than any of the described diseases caused by *F. graminearum*.

The Brazilian soybean isolates are genotypically different from those previously reported on soybean in the U.S.

Recent surveys of U.S. grains have found strains of *F. graminearum* complex lineage 7 exclusively; lineages 1, 2 and 8 have not been found in the U.S. to date (Kistler unpublished). While a recent survey of strains on cereals from Brazil suggest that lineage 7 may be the predominant lineage there, all Brazilian isolates from soybean tested thus far belong to lineages 1, 2 or 8 (Table 1 and unpublished). It will be intriguing to determine if lineage 7 strains can be recovered from soybean in Brazil or whether the other lineages may have made a recent adaptation for causing disease on soybean. However, this hypothesis must be tempered by the fact that, at least in greenhouse tests, U.S. (lineage 7) strains of the fungus also can cause symptoms of disease on soybean and the Brazilian strains from soybean can cause Fusarium head blight on wheat.

Another factor which distinguishes most Brazilian soybean strains from those of *F. graminearum* lineage 7 in the U.S. is the ability of the former, with the exception of strain NRRL 31323, to produce nivalenol during plant infection. Nivalenol and DON production are not strictly correlated with the *F. graminearum* complex lineages (Rodrigues Filho *et al.*, 2002), and the relative importance of these two trichothecenes in plant pathogenesis is currently unknown. The other feature that distinguishes the Brazilian lineage 2 and 8 strains is the production of a novel trichothecene in wheat identified as 3-acetylnivalenol. We do not yet know how widespread production of 3-acetylnivalenol may be among nivalenol producing *Fusarium* species, but future screening of strains for these compounds should take into account the potential for production of this novel trichothecene mycotoxin.

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