Fungi associated with pods and seeds during the R6 and R8 stages of four soybean cultivars in southwestern Indiana

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A total of 6,403 isolates of fungi were identified from soybean pods and seeds collected late in the 1992 and 1993 growing season (R6 and R8 soybean growth stages). The majority of fungi consisted of Deuteromycetes (95.5%) followed by Ascomycetes (0.9%). Common fungal genera isolated during the study included *Phomopsis*, *Alternaria*, *Cercospora*, and *Colletotrichum* (= *Glomerella*). *Cercospora* and *Phomopsis* were identified more commonly from pods and seeds at harvest maturity (R8) than at the greenbean stage of development (R6). However, isolation frequencies of *Colletotrichum* were greater from tissues collected at R6 than at R8. Isolation frequencies compared between pod and seed tissue were similar for almost all the fungi except *Alternaria*, *Phoma*, and *Nigrospora*. The primary pathogenic species identified from the *Diaporthe/Phomopsis* complex were *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* at 28.2% of the total isolation frequencies compared to *D. phaseolorum* var. *meridionalis* and *Phomopsis longicolla* that were identified from 1% of the total samples. The pod tissue harbored greater numbers of fungi than seeds during this study. In statistical comparisons of the peduncle, middle, and stylar regions from pods, no differences in isolation frequencies were found for the cultivars tested regardless if pod tissues or seeds were compared. In summary, the percent isolation frequency of pathogenic fungi from pod and seed at R6 was an effective indicator of the potential for increased disease severity. Furthermore, the significantly greater occurrence of *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* compared to the other *Phompsis/Diaporthe* spp. (e.g. *D. phaseolorum* var. *meridionalis*) in southern Indiana will enable scientists to continue to concentrate their breeding efforts for resistance to control these two major pathogens.

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[Champignons associés aux gousses et aux graines de quatre cultivars de soja aux stades R6 et R8 dans le sud-ouest de l'Indiana]

Un total de 6403 isolats de champignons ont été récupérés de gousses et de graines de soja récoltées vers la fin des saisons 1992 et 1993 (stades de croissance R6 et R8). La majorité des champignons étaient des Deutéromycètes (95,5 %) puis des Ascomycètes (0,9 %). Les genres de champignons les plus communément isolés lors de l'étude furent les *Phomopsis*, *Alternaria*, *Cercospora*, et *Colletotrichum* (=*Glomerella*). Les *Cercospora* et les *Phomopsis* ont été retrouvés plus souvent sur les gousses et les graines à pleine maturité (R8) qu'au stade de développement fève verte (R6). Par contre, la fréquence d'isolement des *Colletotrichum* était plus élevée pour les tissus récoltés au stade R6 que pour ceux au stade R8. Les fréquences d'isolement étaient similaires pour les tissus des gousses ou des graines pour la plupart des champignons sauf les *Alternaria*, *Phoma* et *Nigrospora*. Les principales espèces pathogènes isolées du complexe *Diaporthe*/*Phomopsis* étaient le *D. phaseolorum* var. *caulivora* et le *D. phaseolorum* var. *sojae* avec des fréquences d'isolement totalisant 28,2 % alors que le *D. phaseolorum* var. *meridionalis* et le *Phomopsis longicolla* étaient identifiés dans 1 % de tous les échantillons. Dans cette étude, les tissus des gousses contenaient un plus grand nombre de champignons que les graines. Des comparaisons statistiques des sections du pédoncule, du centre et du style des gousses n'ont pas permis de trouver de différence entre les fréquences d'isolement pour les cultivars testés, que ce soient les tissus des gousses ou les graines qui soient comparés. En résumé, la fréquence d'isolement de champignons pathogènes de gousses et de graines au stade R6 a été un indicateur fiable du potentiel de gravité des maladies. De plus, la présence significativement plus grande du *D. phaseolorum* var. *caulivora* et du *D. phaseolorum* var. *sojae* par rapport aux autres *Phomopsis*/ *Diaporthe* spp. (e.g. *D. phaseolorum* var. *meridionalis*) dans le sud-ouest de l'Indiana va permettre aux scientifiques de continuer à concentrer leurs efforts d'amélioration génétique sur la résistance à ces deux importants agents pathogènes.

INTRODUCTION

A diverse group of organisms, including bacteria and fungi, colonize pods and seeds of soybean plants (*Glycine max* (L.) Merr.) (Ellis *et al.* 1974; Sinclair 1991, 1992, 1993). Fungi isolated from these tissues are classified as saprophytes or parasites (Kulik 1984; Rothrock *et al.* 1985; Sinclair 1991). The parasites or plant pathogens are responsible for millions of dollars lost annually in the north central and southeastern regions of the United States (Doupnik 1993; Scuimbato 1993). The most prevalent pathogens on pods and seeds consist of the *Diaporthe/Phomopsis* spp. complex and *Cercospora kikuchii* (Matsumoto and Tomoyasu) Gardener (Hol-

land and Abney 1988; Roy and Abney 1976; Wilcox and Abney 1973). Fungi within this complex are responsible for pod and stem blight, stem canker, or seed decay, and these fungi cause the greatest loss of seed quality in Illinois (Kmetz *et al.* 1974, 1978; Sinclair 1988, 1993). The primary pathogens are *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *sojae* Wehn. (Dps) responsible for pod and stem blight and *D. phaseolorum* (Cke. and Ell.) Sacc. var. *caulivora* Athow and Caldwell (Dpc) responsible for stem canker. The latter variety has two biotypes associated with either the north central or southeastern regions of the United States (Sinclair 1988). Isolations of Dpc from the southeastern U.S. are referred as the "southern" biotype by Hobbs and Phillips

(1985) and the "northern" biotype predominates in the north central states. More recently, the "southern" biotype has been named *Diaporthe phaseolorum* var. *meridionalis* F. A. Fern. based on morphological characters and the Random-Amplified Polymorphic DNA (RAPD) technique (Fernandez and Hanlin 1996). Holland and Abney (1988) routinely isolated Dps, the northern biotype of Dpc and *C. kikuchii*, the purple seed stain fungus from pods and seed collected in central Indiana. The southern biotypes were isolated infrequently during this investigation. In the southeastern portion of Indiana where climatic conditions are similar to southern states, such as Kentucky and Tennessee, different fungi such as *Glomerella* (*Colletotrichum*), *C. kikuchii*, or the southern var. *sojae* of Dpc may be more important in pod and seed diseases of soybean. Few data are available to substantiate this theory. Knowledge of the major pathogens of soybean pod and seeds present in southern Indiana is important, particularly for the development of soybean varieties with improved resistance to the disease.

Isolation frequencies of fungi from living plant material differ compared to senescing or dead tissues of crops such as soybean and peanut (Baird *et al.* 1993; Holland and Abney 1988). For soybeans, the isolation frequencies of fungal pathogens in the greenbean stage (growth stage R6) may vary compared to mature pods (growth stage R8). The relative occurrence of the prevalent pathogens must be obtained to determine the importance of these fungi in tissues prior to harvest maturity. Latent infections of R6 pods by *Phomopsis* spp. have been shown to spread to immature and mature seeds (Roy and Ratnayake 1997). In particular, only susceptible genotypes have increased seed infections between R7 and R8, especially when pod and seed moisture decreases (Ploper *et al.* 1992). Percent infections of pods by *Phomopsis* spp. during R6 have been reported with greater levels of seed infections at soybean growth stage R8 (McGee 1986; TeKrony *et al.* 1985). Additionally, *Fusarium pallidoroseum* (Cooke) Sacc.

(Syn. *F. semitectum* auct. non Berk. and Ravenel), which attacks soybean pods and seed, was positively correlated with pod infections at R6 to seed infections at R8. The occurrence of *Phomopsis* spp. and other fungal pathogens between pod and seed infections at R6 and R8 growth stages suggests several approaches for disease monitoring (Roy and Ratnayake 1997). The presence of these fungi at R6 as an indicator of disease severity becomes important especially if foliar fungicides might be considered for control. Application of fungicides at R6 significantly reduces seed infections, but if applied at R7, control of the *Phomopsis* spp. is minimal (TeKrony *et al.* 1985). Also, the relative incidence of the fungus in pods of different soybean cultivars is a measure of their relative susceptibility to seed infection. Therefore, it is possible to use R6 through R8 growth stages to screen for resistance (Roy *et al.* 1994) and to elucidate the nature of resistance to pod and seed pathogens, in particular to *Phomopsis* spp. In addition, the use of R6 as a predictive measurement of future seed infection has been successful to predict when fungicides sprays should be used.

Tillage practices can influence disease levels on different crops (Sumner *et al.* 1986). In one study, reduced tillage and double cropping of wheat with soybean significantly increased southern stem canker (Rothrock *et al.* 1985, 1988). Plots that were no-tilled had the greatest disease levels and lowest yields compared to conventional tilled plots (Holland and Abney 1988). Colonization of soybean stems and pods by *Diaporthe* spp. was more prevalent in continuous soybeans under minimal tillage (Abney and Richards 1982).

Many of the pathogens that infect pods and seeds can go undetected without any obvious symptoms (McGee 1992). If the diseases are expressed, the seed is often discolored, split, or shrunken and light in weight. Genera of fungi that cause latent infections and are asymptomatic included *Cercospora*, *Colletotrichum*, *Diaporthe* (*Phomopsis*), *Fusarium*, and *Macrophomina* (Glawe 1989). The amount of yield loss

caused by these fungi in southern Indiana is unknown and further studies are necessary to determine their economic impact.

The objectives of the study were to determine the biodiversity of the common pathogens of pods and seeds in southwestern Indiana and to compare differences in isolation frequencies from pods collected at R6 and R8 soybean growth stages.

MATERIALS AND METHODS

Soybean cultivars Fayette, Ripley, Spencer, and Williams 82 used in this investigation were obtained from two locations in 1992 and a third location in 1993. The trials were located at the Primus Farm, Vincennes, IN and the Uhde Farm, Mt. Vernon, IN in 1992. The third site, at the Schnur Farm, Evansville, IN included the cultivars Ripley, Spencer, and Williams 82. At the three locations, the trials were conducted with a randomized complete block design with four replicates per cultivar.

Experimental sites

Primus Farm
In 1992 and 1993, two separate but adjacent fields were used. The first trial had a corn-soybean (c-s) rotation prior to 1992. Tillage practices included chisel-plowing and disking twice prior to planting. The field used in 1993 had continuous corn (c-c) the two previous seasons and no-tillage practices were employed in 1993. Approximately 134.5 kg ha^{-1} K and 39 kg ha^{-1} P were applied to both fields prior to planting soybeans. Each cultivar plot was three rows (2.3 m x 3.8 m) with 0.8 m centers at both trials. The four soybean cultivars were planted on 28 April 1992 and 25 May 1993 on an Elston sandy loam. All cultural practices and pest control methods were conducted according to state recommendations. The field received a total of 11.1, 4.1, 11.3, 7.7, and 12.3 cm of rain in May, June, July, August, and September, respectively, in 1992 and 11.1, 3.2, 9.9, 10.0, and 20.9 cm, respectively, in 1993.

Twenty three-seeded pods (10 plot^{-1}) randomly selected in the cultivar plots were hand harvested twice during each growing season. The first soybean samples, consisting of pods in the green-bean stage (R6), were harvested on 31 August, and dry pods (R8), on 15 October in 1992. During each sampling date for both yr, pod samples at R6 for each location were immediately processed, but the R8 samples were stored in paper packets at room temperature up to 72 h. In 1993, R6 pods were collected on 7 September and R8 pods on 15 October.

Uhde Farm
The field used in the 1992 trial was planted in soybean-corn (s-c) the two preceding yr. The field was disked twice and 123.3 kg ha^{-1} K, 72 kg ha^{-1} P, and 16.8 kg ha^{-1} N were applied each yr prior to planting. Each plot was three rows (2.3 m x 3.8 m) with 0.8 m centers. The four cultivars were seeded on an Alford silt loam soil 3 May 1992 and 23 May 1993. The field received a total of 10.9, 4.3, 11.7, 7.5, and 10.5 cm of rain in May, June, July, August, and September, respectively, for 1992 and 11.2, 3.2, 9.9, 10.6, and 20.9 cm in 1993.

Methods of collection of R6 and R8 pods were the same as described previously for the Primus Farm. The R6 samples were collected on 9 September and R8 on 2 October, in 1992. In 1993, R6 were collected on 22 September and R8 on 15 October.

Schnur Farm
The field trial established only in 1993 had been planted in soybean-corn rotation the two preceding yr. The field was disked twice and 224 kg ha^{-1} K and 168 kg ha^{-1} P were applied prior to planting. Each cultivar plot was four rows (3.0 m x 6.0 m) with 0.8 m centers. The soybeans were seeded 11 May 1993 on an Evansville silt loam soil. The field received a total of 7.6, 11.5, 9.8, 3.0, and 17.0 cm of rain in May, June, July, August, and September, respectively. Pod samples at the R6 growth stage were hand harvested on 23 September and R8 on 16 October. These pod and seed samples were processed using the methods previously described.

Laboratory procedures

Each three-seeded pod was split down the sutures and with a paper punch, 0.5 cm pod discs were removed from the peduncle (P), the middle (M), and the stylar (S) areas from one-half of each carpel. Pod discs (ten each of P, M, and S) and seeds for each soybean cultivar were disinfected in 9% ethanol for 30 s and immediately placed in 0.525% (w:v) aqueous sodium hypochlorite solution for 1 min, and then plated onto potato dextrose agar (PDA) (Baird *et al.* 1991). Fungi that grew from the tissue were identified and recorded per P, M, and S positions. Percent frequencies were determined for the genera and species isolated. Single spores of cultures initially identified as *Fusarium* spp. were transferred to carnation leaf agar and identified by the classification system of Nelson *et al.* (1983). Keys for general identification of the fungi were those developed by Ellis (1971), Sutton (1980), and Barnett and Hunter (1998).

Statistical analysis

Percent isolation frequencies of the fungi were evaluated using an analysis of variance to identify significant ($P < 0.05$) interactions among means. The Baysian least significant difference test (BLSD) was used to evaluate k-ratios at $P < 0.05$.

RESULTS AND DISCUSSION

A total of 6,403 isolates of fungi were obtained from pods at R6 and R8 and from seed. Among these isolates, 95.0% of the fungi were Deuteromycetes followed by Ascomycetes at 0.9% (primarily of a *Colletotrichum* [= *Glomerella* spp.]), and the remaining classes of fungi were isolated from ≤ 1% of all tissue types (Table 1). In a study on the survival of soybean pathogens on no-tillage debris, over 90% of all isolations were also Deuteromycetes (Baird *et al.* 1997).

The isolation frequencies of the fungi were similar between the P, M, and S positions of pod tissue (data not shown). Futhermore, the frequencies of the fungi from seed at the three pod positions were also similar. Initial fungal infections were not site specific on the pods, but occurred randomly based on where the spores were deposited during dissemination. Knowing that soybean pathogens occur randomly on the pod tissue is important, especially to determine if fungicide sprays should be recommended for use to prevent seed damage and yield loss prior to R8.

The most commonly isolated genera from the R6 and R8 tissues were *Phomopsis* and *Diaporthe* that consisted of *Diaporthe phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora*, which comprised 28.2% of the total isolations (Table 1). Other commonly isolated genera included *Alternaria* (27.9%), *Cercospora* (15.6%), *Colletotrichum* spp. (6.6%), and *Phoma* (4.9%). The common genera present on soybean pods and stems were *Alternaria, Cercospora, Epicoccum, Fusarium, Phoma*, and *Phomopsis* (Baird *et al.* 1997; Schmitthenner 1989).

Comparisons of isolation frequencies of the fungi from the pooled tissue data were similar between R6 and R8 growth stages. These frequencies were compared between pod or seed tissues excluding the ones discussed in Tables 2 and 3. Furthermore, no differences in percent frequencies were observed between the three locations in the pods (e.g. stylar region).

Cercospora kikuchii was isolated more frequently from seed than pod tissues over both yr (data not shown). Furthermore, the percent isolations from R8 tissues were numerically or significantly greater than at the R6 stage, but no consistent trends were noted among the four cultivars and locations (Table 2). In 1992, however, *C. kikuchii* was isolated from less than 1% of pods and seeds tissues from 'Williams 82' and no differences in frequency of isolations could be determined between the two soybean growth stages. *Cerospora kikuchii* annually causes an average of 2% crop loss in the Midwest. The Cercospora leaf blight phase of the disease can potentially be more important under certain environmental situations (Rupe 1989). Since *C. kikuchii* was shown to occur in southern Indiana,

Table 1. Frequency of fungi isolated from soybean pod and seed samples[a] in southwestern Indiana

Fungi	1992 Pod and seed samples		1993 Pod and seed samples	
	R6 %	R8 %	R6 %	R8 %
Deuteromycetes				
Alternaria spp.	[b]28.0	33.0	27.0	23.7
Cephalosporium sp.	0	0	0.1	0
Cercospora kikuchii	2.1	12.6	14.6	33.2
Cladosporium sp.	1.5	0.1	1.5	1.1
Epicoccum nigrum	4.2	0.7	1.4	1.4
Fusariella sp.	0	0	0.1	0
Fusarium equisiti	1.5	2.8	0	0.1
F. graminearum	1.7	1.0	0.6	0.7
F. moniliforme	0.6	1.0	0	0.1
F. semitectum	0.2	2.8	2.6	2.1
F. solani	0	0.3	1.2	2.4
Fusarium spp.	0	0.3	0.1	0.2
Gliocladium sp.	0	0	1.7	0.3
Graphium sp.	0	0	1.0	0
Humicola sp.	0	0	0.2	0.4
Helicorhoidon sp.	0.2	0	0	0
Macrophoma sp.	0	0	0.2	0
Nigrospora sp.	14.2	0	0.8	0.4
Nodulosporium sp.	0.3	0.6	0.6	0
Paecilomyces sp.	0.1	0	0.1	0
Penicillium oxalicum	0	0.3	1.0	0.2
Penicillium sp.	0	0	0.1	0
Phialophora sp.	0	0	0.9	0.2
Phoma spp.	14.3	5.0	0.2	0.1
Phomopsis (*Diaporthe*)[c]	26.3	38.9	22.1	25.6
Pithomyces sp.	0.7	0.1	0.1	0
Sclerococcum sp.	0	0	0.1	0
Septoria glycines	0.8	0	0.1	0
Sphaeropsis sp.	1.0	0	0	0
Ulocladium sp.	0	0	0.1	0
Verticillium sp.	0	0	1.2	0.5
Unknown	1.0	0	0.3	0.3
Ascomycetes				
Chaetomium spp.	0.6	0.3	1.4	0.1
Glomerella glycines	0.2	0	19.1	7.0
Leptosphaeria sp.	0.4	0.6	0.1	0
Xylaria sp.	0	0	0.1	0
Basidiomycetes				
Unknown	0	0	0.4	0.1
Zygomycetes				
Pythium sp.	0	0	0.3	0
Rhizopus sp.	0.4	0	0	0

[a] In 1992, a total of 1,926 fungi were isolated from R6 (greenbean stage) and 722 from R8 (harvest maturity stage) soybean samples. In 1993, 1,448 fungi were isolated from R6 and 2,307 fungi from R8 soybean samples.

[b] Percentage for fungi isolated from soybeans at two southwestern Indiana farms in 1992 and at three farms in 1993.

[c] *Diaporthe phaseolorum* var. *sojae* was isolated from 22.2% of the tissues and *D. phaseolorum* var. *caulivora* from 5%; *D. phaseolorum* var. *meridionalis* and *Phomopis longicolla* from 1% combined.

Table 2. Frequency of selected fungal pathogens isolated from soybean cultivars

Fungi	Cultivars[a]			
	Fayette %	Williams 82 %	Ripley %	Spencer %
Cercospora kikuchii				
1992 R6 pods & seed	1.3 B[b]	0.2 A	5.0 B	1.9 B
R8 pods & seed	5.4 A	0.1 A	13.8 A	8.8 A
1993 R6 pods & seed	5.2 A	9.5 B	14.8 B	9.5 B
R8 pods & seed	31.2 A	31.4 A	33.9 A	30.8 A
Diaporthe/Phomopsis				
1992 R6 pods & seed	26.0 B	11.9 B	31.5 A	35.8 A
R8 pods & seed	44.2 A	32.7 A	36.3 A	36.7 A
1993 R6 pods & seed	16.0 A	11.2 A	9.5 A	11.5 A
R8 pods & seed	23.1 A	24.4 A	14.1 A	15.0 A
Glomerella/Colletotrichum				
1992[c]				
1993 R6 pods & seed	16.7 A	10.5 A	17.9 A	9.5 A
R8 pods & seed	7.1 A	6.2 A	8.3 A	5.3 A

[a] 'Fayette' and 'Williams 82' are Maturity Group III soybeans; 'Ripley' and 'Spencer' are Maturity Group IV soybeans; percentages for fungi isolated from soybeans at two southwestern Indiana farms in 1992 and at three farms in 1993; all four cultivars evaluated both years, but 'Fayette' was not included at one location in 1993.

[b] Comparison of cultivar percent means for R6 and R8 samples and a given year followed by the same letter do not differ significantly (k ratio = 100) according to the Bayesian LSD test.

[c] Insufficient data to analyze.

Table 3. Frequency of *Alternaria, Nigrospora,* and *Phoma* species isolated from pods and seed of four soybean cultivars

Fungi		Cultivars[a]			
		Fayette %	Williams 82 %	Ripley %	Spencer %
Alternaria spp.					
	1992 seed	7.8 B[b]	5.4 B	10.0 B	8.9 B
	pods	58.1 A	36.3 A	41.4 A	62.8 A
	1993 seed	4.3 B	7.7 B	7.4 B	9.1 B
	pods	31.0 A	35.5 A	22.4 A	42.9 A
Nigrospora sp.					
	1992 seed	15.6 A	35.4 A	13.6 A	14.7 A
	pods	1.1 B	9.6 B	0.8 B	0.1 B
	1993[c]				
Phoma spp.					
	1992 seed	0.8 B	11.7 B	5.8 B	1.4 B
	pods	17.2 A	35.4 A	16.7 A	13.3 A
	1993[c]				

[a] 'Fayette' and 'Williams 82' are Maturity Group III soybeans; 'Ripley' and 'Spencer' are Maturity Group IV soybeans; percentages for fungi isolated from soybeans at two southwestern Indiana farms in 1992 and at three farms in 1993; all four cultivars evaluated in both years, but 'Fayette' was not included at one location in 1993.

[b] Comparison of cultivar percent means for pod and seed samples and a given year followed by the same letter do not differ significantly (k ratio = 100) according to the Bayesian LSD test.

[c] Insufficient data to analyze.

development of resistant cultivars to the pathogen would be important for soybean producers in that portion of the state. The occurrence of *C. kikuchii* or other pathogens during R6 stage may be a good indicator to determine increased disease (Roy *et al.* 1994). Fungicides, if economical, may be used by growers to control the pathogen and limit yield loss from pod and seed damage. These chemicals must be applied prior to the R7 for maximum benefit.

Isolation frequencies of the fungi in the *Diaporthe* spp. (=*Phomopsis*) complex were greater during R8 than R6 from both tissue types (Table 2). *Diaporthe phaseolorum* var. *sojae* (Dps) was commonly isolated from soybean pod and seed tissues in 1992 and 1993 (Table 2). There were significantly greater frequencies at the Primus Farm in 1992 from the four cultivars, and for 'Williams 82' at the Primus and Schnur Farms in 1993. In northern Indiana, Dps and *D. phaseolorum* var. *caulivora* (Dpc) were previously reported to cause economic yield losses of soybean. In this study, however, Dps was believed to be the principal *Diaporthe* spp. identified from 22.2% and Dpc from 5% of soybean tissues in this investigation. Other members of the *Diaporthe* spp. complex such as the southern stem canker pathogen, *Diaporthe phaseolorum* var. *meridionalis*, and *Phomopsis longicolla* Hobbs were identified, but only from 1% of the soybean tissues in this study. Isolation frequencies of Dps were greater at R8 than R6, but no significant differences occurred between the pod and seed tissues (data not shown). Isolation frequencies of Dps were significantly greater at R8 for all cultivars in 1992 at the Primus Farm, but only from the pod and seed tissues of 'Williams 82' collected from the Primus and Schnur Farms in 1993. Even though the isolation frequencies of the fungi were greater at R8 than R6 across all tissue types, results between the pods and seeds were similar (data not shown). Pod and seed development from growth stages R6 and R7 can occur between 9-30 d depending on plant growth conditions (Fehr and Caviness 1977). Therefore, if Dps is present in the R6 stage, fungicide application must occur prior to the R7 stage to arrest any further disease development (TeKrony *et al.* 1985).

The two varieties of *Diaporthe phaseolorum*, Dps and Dpc, identified in this investigation are responsible for pod and stem blight and northern stem canker. Previously, *Diaporthe* spp. were reported to be responsible for over 15% soybean crop loss in the Midwest (Doupnik 1993) and 17.5% loss in the southeast (Sciumbato 1993).

Colletotrichum truncatum was commonly isolated from pods and seeds of the four cultivars over all three locations in 1993, but the frequency was low (0.2%) from both tissue types in 1992 (Table 2). During 1993, when higher level of moisture occurred late in the growing season, the pod tissue at R6 supported increased colonization of *Colletotrichum/Glomerella* than at R8 stage. Crop loss by this pathogen that causes anthracnose can exceed 19% in the Midwest (Doupnik 1993). Isolation frequencies of *C. truncatum* were greater at R6 than at R8. When the isolation frequencies were compared between the seed and pod tissues, no differences were observed (data not shown). *Colletotrichum truncatum* has been identified on soybeans in northern Indiana in previous studies, but the incidence or occurrence was low (Abney unpublished data). In this study, *C. truncatum* can be an important pathogen in southern Indiana, but disease potential varies by yr. The greater isolation frequencies of *C. kikuchii* and Dps at R8 than at R6, means that these fungi may be better competitors than *C. truncatum* for substrate or that they may be antagonistic to *C. truncatum*.

When fungi were isolated from seeds and pods, significant differences were observed for *Alternaria* spp., *Nigrospora* spp. and *Phoma* spp. (Table 3). *Alternaria* spp., which were identified primarily as *Alternaria alternata* and *Alternaria tenuissima*, were always isolated at numerically and significantly greater frequencies from pods than seed tissues, but the differences varied during each yr of the study (Table 3). Both *Alternaria* spp. identified in the study have been reported to cause late season pod and seed decay (Sinclair 1999),

however, crop losses are believed to be minimal from this fungus. *Alternaria* spp. can occur on soybean plants grown in northern Indiana, but the economic loss from these fungi is unknown (Abney and Ploper 1988).

Nigrospora spp. occurred more frequently on seeds than pods at both locations and across the four cultivars in 1992. However, *Phoma* spp. was isolated primarily from pods (Table 3). *Nigrospora* and *Phoma* spp. were previously reported to occur on soybeans, however, no documented evidence is available to show that these fungi affect seed quality or result in losses due to disease (Kulik and Sinclair 1999). Roy (1976) confirmed that *Nigrospora* spp. and *Phoma* spp. occurred on soybean plants grown in northern Indiana, but their economic importance was not determined.

Data were pooled across the five *Fusarium* spp. identified in this study and compared between the R6 and R8 growth stages (Table 1). Isolation frequencies from all locations and cultivars were almost always similar between R6 and R8. The frequencies at R8 were significantly greater than at R6 for the cultivars, but the percentages varied per cultivar and yr with no specific trends observed. In northern Indiana, *Fusarium* spp. can be commonly isolated from pod and seed tissues (Abney and Ploper 1988), but there was no discussion of these fungi as to their importance as pathogens of soybeans or for other crops.

Isolation frequencies of *Fusarium* spp. were almost always numerically greater on seeds than pods over both yr (data not shown). For a few exceptions, percent frequencies were greater in pods than seeds, but these differences varied by cultivars and years without any obvious trends. Fusarium pod and collar rot, caused by *Fusarium pallidoroseum* was reported from India (Nelson 1999) and was identified from the soybean tissues across both yr at 0.3% frequencies. From the five common *Fusarium* spp. identified in this study (Table 1), only *F. solani* (not the SDS

pathogen), *F. equisiti*, and *F. graminearum* have been reported to be pathogenic on soybeans (Nelson 1999), but the incidence and disease losses are unreported for Indiana.

The percent isolation frequencies for total fungi varied between R6 and R8 with no consistent patterns in frequencies being observed for specific cultivars (data not shown). In most cases frequencies of isolation in the R6 and R8 stages were similar. At the Primus Farm in 1992, isolation frequencies were significantly greater at R6 than R8 for 'Fayette' and 'Ripley', and numerically greater for 'Spencer'. It was uncertain why frequencies decreased at this location.

Isolation frequencies of the fungi were compared between pod and seed tissues and the results were somewhat inconsistent for species of *Glomerella/ Colletotrichum*, *Phomopsis*, and *Cercospora*. *Alternaria* was consistently isolated at a significantly greater frequency from pod tissues for each yr of the study (Table 3). *Phoma* was isolated at a significantly greater level from pod tissues for each cultivar in 1992, but almost no collections of this fungus were obtained in 1993. *Nigrospora* and *Phoma* were previously reported to be seedborne on soybean, but their potential as pathogens was questioned (Schmitthenner 1989). The isolation frequencies for total fungi were significantly greater from pod tissues than from seeds for each cultivar during both yr. Soybean debris would be critical to increase the pathogen population at planting if continuous soybean cropping in a no-tillage production field is practiced.

For total fungi identified in this study, isolation frequencies were almost always greater from pods than seeds (data not shown). Furthermore, these differences in isolation were significantly greater from pods across all cultivars sampled at the Schnur Farm in 1993. These results indicate that pod tissue alone is not a good indicator of seed colonization by saprophytic or parasitic fungi.

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