Soybean Cultivar and Foliar Fungicide Effects on *Phomopsis* sp. Seed Infection

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ABSTRACT

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Phomopsis seed decay (PSD) caused by *Phomopsis* spp. can be severe when soybean seed producers in the southern United States use the early soybean production system (ESPS) to avoid late-July through early-September drought damage to soybean. The usefulness of this production system would be greater if developing seed could be protected from PSD by foliar application of fungicides or by planting *Phomopsis* spp.-resistant soybean lines. The objective of this research was to determine the affects of the fungicides benomyl and azoxystrobin applied to soybean, at various times, on percent *Phomopsis* spp. infection of seed in Asgrow 3834, a PSD-susceptible cultivar, and SS93-6012, a PSD-resistant soybean line, planted in mid-April. The percent *Phomopsis* spp. infection of Asgrow 3834 seed averaged over years was significantly less for the benomyl (0.28 kg a.i. ha⁻¹) applied at R3 + R5 treatment (48.6% seed infection) than the control (52.8% seed infection) and significantly greater for the azoxystrobin (0.17 kg a.i. ha⁻¹) applied at R3 + R5 treatment (61.6% seed infection) than the control (52.8% seed infection). This method of managing PSD will not be acceptable to soybean growers. The percent of *Phomopsis* spp. infection of Asgrow 3834 seed averaged over years (52.8% seed infection) was significantly greater than for line SS93-6012 (2.8% seed infection). There were no differences in percent *Phomopsis* spp. infection of SS93-6012 seed between the control (2.8% seed infection) and benomyl treatment (4.0% seed infection). The most effective method for PSD management was to plant a resistant soybean line. Line SS93-6012 will be useful in breeding programs focused on developing high yielding PSD-resistant cultivars.

The primary cause of *Phomopsis* seed decay (PSD) is *Phomopsis longicolla* T. W. Hobbs, but other species may be involved (11). This disease is endemic throughout most soybean-growing states in the United States (11), but incidence and severity of this disease varies among years and among regions (16) of the United States due to differences in weather at harvest. PSD is greater when harvest is delayed due to wet weather or when seed mature during mid- to late summer rather than in early fall (15).

Some soybean growers in the southern United States have adopted a soybean production system that results in seed maturing during mid- to late summer. This system, early soybean production system (ESPS), was designed to help growers in the south avoid late-July through early-September drought damage to soybean (4).

Success of this system is dependent on planting early-maturing cultivars (MG III to IV) earlier than normal (early- to late-April rather than mid-May to mid-June), so that pod fill is mostly complete before late-summer droughts develop. Yield advantages of this system have been observed in Arkansas (7), Mississippi (4), and Texas (8).

Yield advantages for this system have been observed in southeast Missouri (15,17), but only during years when rainfall is abundant. Unfortunately, PSD was severe in some MG III and IV cultivars planted during mid-April in Arkansas (7) and Missouri (15,17). This disease has suppressed yield, reduced the quality of seed used for planting (by reduced seed germination), and lowered the quality of seed used for processing (10,15). The quality of oil derived from seed infected with *Phomopsis* spp. was lower than that from healthy seed (5), due to altered palmitic, oleic, linoleic, and linolenic acid composition (17). Usefulness of ESPS would be greater if seeds could be protected from PSD either by foliar application of fungicides or by planting resistant cultivars. Unfortunately, PSD-resistant cultivars currently are not commercially available.

Foliar applications of some rates of benomyl to soybean at various growth stages have reduced the percentage of seed infected by *Phomopsis* spp. and *Diaporthe phaseolorum* var. *sojae* (S. G. Lehman)

Wehmeyer. Tekrony et al. (14) and Ellis et al. (2) determined that a single, R6 (3) application of benomyl (0.28 or 0.56 kg a.i. ha⁻¹) to soybean foliage of soybean planted in mid-May reduced the percent of *Phomopsis* spp.- and *D. phaseolorum* var. *sojae*-infected seed, respectively, compared with controls. However, a single, R7 (3) application of benomyl (0.28 or 0.56 kg a.i. ha⁻¹) to foliage of soybean planted in mid-May did not reduce the percent *Phomopsis* spp. infection of seed (6,14). Effects of foliar-applied fungicides on percent *Phomopsis* spp. infection of seed of early-planted (mid-April rather than mid-May), early-maturing soybean is not known. The objective of this research was to determine the affects of the fungicides benomyl and azoxystrobin applied to soybean foliage on percent *Phomopsis* spp. infection of mid-April-planted Asgrow 3834 seed, a PSD-susceptible cultivar, and SS93-6012, a PSD-resistant soybean line. Soybean line SS93-6012 was developed from a cross of a PSD-resistant line, MO/PSD-0259, and Asgrow 3322 (17). Benomyl currently is not labeled for use on soybean but was included in this study to provide some continuity with previously published reports. Azoxystrobin currently is labeled for foliar application to soybean.

MATERIALS AND METHODS

Experimental plot management. Plots for two experiments were established in 2000 and 2001 at a research center near Portageville, MO on a silt loam soil consisting of 56.5% sand, 37.2% silt, and 6.3% clay. The field had been planted to soybean during 1998 and 1999. One experiment (experiment 1) was designed to determine the effects of various application timings of benomyl and azoxystrobin to foliage on percent *Phomopsis* spp. infection of Asgrow 3834 seed. The second experiment (experiment 2) was designed to determine the effects of applications of benomyl and azoxystrobin to foliage at growth stages R3 and R5 on percent *Phomopsis* spp. infection of Asgrow 3834 and soybean line SS93-6012. The experiments were in the same field each year. Prior to planting each year, the field was disked twice, and row beds (75-cm spacing) were formed. The top 10 cm of the beds were pushed off just prior to planting to form a flat-top ridge. Soybean cv. Asgrow 3834 (MG III) and line SS 93-6012 (MG III) were planted on 21 April 2000 and 15 April 2001. Each four-row plot was 3 m

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Table 1. Analysis of variance for *Phomopsis* spp. infection of Asgrow 3834 for years and foliar fungicide treatments

Source of variation ^z	<i>P</i> > <i>F</i> seed infected (%)
Year (Y)	0.0089
Fungicide treatment (T)	0.0159
Y × T	0.6906

^z Years were 2000 and 2001. Fungicide treatments were control (water only), benomyl (0.28 kg a.i. ha⁻¹), and azoxystrobin (0.17 kg a.i. ha⁻¹) applied at various growth stages.

wide and 7.6 m long, and 26 seed were planted per meter of row. All plots received a pre-emergence application of imazaquin (BASF Corporation, Agriculture Products, Research Triangle Park, NC) at 0.14 kg a.i. ha⁻¹ and alaclor (Monsanto Co., Agriculture Group, St. Louis) at 0.4 kg a.i. ha⁻¹. Plots were cultivated twice and hand weeded each year as necessary. Plots were located in a different area of the same field each year to avoid problems caused by repeated planting in the same area. Plots were not irrigated. For experiment 1, a randomized complete block design with four replications and nine foliar fungicide treatments was used to evaluate the effects of timings of fungicides on *Phomopsis* spp. infection of Asgrow 3834 seed. For experiment 2, a split plot design was used with four replications, and included two cultivars as main plots and foliar fungicide treatments as subplots, to determine the effects of fungicides on *Phomopsis* spp. infection of Asgrow 3834 and SS93-6012 seed.

Fungicide application. Treatments were sprayed on plants at growth stages R3, R5, and R6 (3). The fungicides and rates applied were benomyl (Benlate, Dupont, Crop Protection Division, Wilmington, DE) at 0.28 kg a.i. ha⁻¹ and azoxystrobin (Quadris, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.17 kg a.i. ha⁻¹. Treatments were applied in water with a CO₂-powered handheld boom sprayer calibrated to deliver 187 liters ha⁻¹ at 207 kPa.

Seed analysis. Each year, the middle two rows of each plot in each experiment were machine harvested at maturity. A 500-g sample of harvested seed from each plot was stored in a paper bag at 25°C and analyzed within 30 days for percentage of seed infected with *Phomopsis* spp. One hundred arbitrarily chosen seed from each plot were surface disinfested in 0.5% sodium hypochlorite for 5 min, rinsed in sterile distilled water, and placed on acidified (pH 4.5) Difco potato dextrose agar in petri dishes (13). Five seed were placed on each culture dish and incubated under fluorescent lights at 25°C. Seedborne fungi were identified based on morphological characteristics 7 days later (1,11).

Data analysis. For both experiments, statistical analyses of fungicide treatment

Table 2. Foliar-applied fungicide effects on percent *Phomopsis* spp. infection of Asgrow 3834 seed averaged over years^y

Fungicides	Time of application ^z	Seed infected (%)
Control	...	52.8 cd
Benomyl	R3	53.6 c
Benomyl	R5	50.6 de
Benomyl	R6	52.4 cd
Benomyl	R3 + R5	48.6 e
Azoxystrobin	R3	62.8 a
Azoxystrobin	R5	54.9 c
Azoxystrobin	R6	58.6 b
Azoxystrobin	R3 + R5	61.6 a

^y Years were 2000 and 2001. Fungicides applied were control (water only), benomyl (0.28 kg a.i. ha⁻¹), and azoxystrobin (0.17 kg a.i. ha⁻¹). Values followed by the same letter are not significantly different (*P* = 0.05).

^z Fungicides were applied at R3 (pod 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf), R5 (beans can be felt when the pod is squeezed at one of the four uppermost nodes with a completely unrolled leaf), and R6 (pods containing full-size green beans at one of the four uppermost nodes with a completely unrolled leaf).

effects on percent *Phomopsis* spp. infection of Asgrow 3834 and SS93-6012 seed were performed using SAS Mixed Model procedures (SAS Institute, Cary, NC). The Mixed Model procedure provides type-III *F* values but does not provide mean square values for each element within the analysis or the error terms. Mean separation was evaluated through a series of protected pairwise contrasts among all treatments (9). Probability levels greater than 0.05 were categorized as nonsignificant. Percent seed infected with *Phomopsis* spp. during 2000 and 2001 in the experiment with Asgrow 3834 and SS93-6012 was transformed before analysis ($X + 0.5$)^{1/2}, where *X* is the original percent infection (17), because the percent *Phomopsis* spp. infection of SS 93-6012 seed from all treatments was very low (<10%) during both years. The letter grouping for the transformed means was applied to the original treatment means.

RESULTS

Analysis of variance for percent *Phomopsis* spp. infection of Asgrow 3834 seed indicated there was no year–fungicide treatment interaction (Table 1), but there was a year effect. The percent *Phomopsis* spp. infection of Asgrow 3834 seed, averaged over fungicide treatments, was significantly greater for 2001 (61.1%) than for 2000 (49.1%). There also was a fungicide treatment effect. The percent *Phomopsis* spp. infection of Asgrow 3834 seed averaged over years was significantly less for the benomyl applied at R3 + R5 treatment than the control, albeit differences were slight (Table 2). There were no differences in *Phomopsis* spp. infection of Asgrow 3834 seed among the other benomyl treatments and the control. The per-

Table 3. Analysis of variance for *Phomopsis* sp. infection of seed for years, cultivars, and foliar fungicide treatments

Source of variation ^z	<i>P</i> > <i>F</i> seed infected (%)
Year (Y)	0.0345
Cultivar (C)	<0.0001
Y × C	0.0149
Fungicide (F)	0.0038
Y × F	0.2008
C × F	0.0025
Y × C × F	0.1425

^z Years were 2000 and 2001. Cultivars were Asgrow 3834 and soybean line SS 93-6181. Fungicide treatments were control, benomyl (0.28 kg a.i. ha⁻¹), and azoxystrobin (0.17 kg a.i. ha⁻¹) applied at R3 + R5 growth stages where R3 = pod 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf and R5 = beans can be felt when the pod is squeezed at one of the four uppermost nodes with a completely unrolled leaf.

cent *Phomopsis* spp. infection of Asgrow 3834 seed averaged over years was significantly greater for azoxystrobin applied at R3, R6, and R3 + R5 than the control.

Analysis of variance for percent *Phomopsis* spp. infection of Asgrow 3834 and SS93-6012 seed indicated that there were significant cultivar–fungicide interactions (Table 3). The percent *Phomopsis* spp. infection of Asgrow 3834 seed was significantly greater for the control and azoxystrobin treatment than the benomyl treatment; moreover, infection of Asgrow 3834 seed was significantly greater than for line SS93-6012 (Table 4). There were no differences in percent *Phomopsis* spp. infection of SS93-6012 seed among fungicide treatments.

Analysis of variance for percent *Phomopsis* spp. of Asgrow 3834 and SS93-6012 seed infection also indicated that there was a significant year–cultivar interaction (Table 3). The percent *Phomopsis* spp. infection of Asgrow 3834 seed was significantly greater for 2001 than 2000 (Table 5). The percent *Phomopsis* spp. infection of Asgrow 3834 seed infected was significantly greater than for line SS 93-6012 both years.

DISCUSSION

Some benefits of ESPS have been shown in Arkansas (12), Mississippi (4), Texas (8), and Missouri (15,17). Early-maturing cultivars (MG III) planted in early to mid-April are ready for harvest about late-August to early September, and this is about 14 days earlier than for mid-May plantings. Earlier harvest is an advantage for those growers who need to extend harvest over a longer period for more efficient use of labor and harvest machinery (15). Yield advantages for mid- to late-April compared with mid- to late-May plantings of soybean have been reported from Texas (8), Mississippi (4), and some Missouri conditions (17), an obvious ad-

Table 4. Cultivar and foliar fungicide treatment effects on percent *Phomopsis* sp. infection of Asgrow 3834 and line SS93-6012 seed averaged over years^z

Fungicide treatment	Seed infected (%)	
	Asgrow 3834	SS 93-6012
Control (water only)	52.8 b	2.8 d
Benomyl (0.28 kg a.i. ha ⁻¹)	49.3 c	4.0 d
Azoxystrobin (0.17 kg a.i. ha ⁻¹)	62.9 a	3.4 d

^z Years were 2000 and 2001. Fungicide treatments were applied at R3 + R5 growth stages. Values followed by the same letter are not significantly different ($P = 0.05$).

Table 5. Cultivar and year effects on percent *Phomopsis* sp. infection of seed averaged over foliar fungicide treatments^z

Years	Seed infected (%)	
	Asgrow 3834	SS 93-6012
2000	48.4 b	3.1 c
2001	61.6 a	3.7 c

^z Foliar fungicide treatments were control (water only), benomyl (0.28 kg a.i. ha⁻¹), and azoxystrobin (0.17 kg a.i. ha⁻¹) applied at R3 + R5 growth stages. Values followed by the same letter are not significantly different ($P = 0.05$).

vantage. Unfortunately, *Phomopsis* spp. infection of seed harvested from MG III cultivars planted in mid-April can be greater than for mid-May plantings (7,15,17). Our results also showed that *Phomopsis* spp. infection of seed can be a problem for a susceptible cultivar (Asgrow 3834) planted in mid-April.

This is the first report on the effect of benomyl and azoxystrobin application to foliage of mid-April-planted MG III cultivars on percent *Phomopsis* spp. infection of seed. Benomyl at 0.28 kg a.i. ha⁻¹ applied at R3 + R5 significantly reduced the percent infected seed compared with the control. However, averaged over 2 years, 48% of the seed of Asgrow 3834 treated with benomyl at R3 + R5 were still infected. Thus, this method of managing PSD will not be acceptable to soybean growers because none of the fungicide treatments tested provided practical levels of disease control.

Greater rates of benomyl, such as 0.56 kg a.i. ha⁻¹, might have further reduced percent *Phomopsis* spp. infection of Asgrow 3834 seed than we observed with 0.28 kg a.i. ha⁻¹. Tekrony et al. (14) determined that foliar application of benomyl at 0.56 kg a.i. ha⁻¹ at R6 to May-planted soybean reduced percent *Phomopsis* spp.

infection of seed more than 50% compared with the control. Jardine, however, showed that 0.28 kg a.i. ha⁻¹ did not effect percentage of *Phomopsis* spp. seed infection compared with the control (6). In any event, benomyl is no longer labeled for use on soybean.

Soybean producers using the ESPS should be aware that foliar application of azoxystrobin to soybean foliage may be useful for management of some foliage diseases, but this product may increase the percent *Phomopsis* spp. seed infection. The reason some azoxystrobin treatments enhanced percent *Phomopsis* spp. infection of Asgrow 3834 seed compared with the control is not known. This product may interfere with the plant's natural defense mechanism to *Phomopsis* spp., or it may protect the plant from other diseases, thus extending the life of the plant so that *Phomopsis* spp. have more time to move through the pod and into seed.

Soybean growers need a high yielding, early-maturity cultivar that is resistant to PSD. Wrather et al. (17) were the first to report the successful management of this disease by planting early-maturing, PSD-resistant soybean lines SS93-6012 and SS93-6181. Our data confirms that report about management of this disease by planting SS93-6012. Line SS93-6012 will be useful in breeding programs focused on developing high yielding *Phomopsis* seed decay-resistant cultivars.

Our plots were harvested when the crop was first mature. If harvest had been delayed, the percent *Phomopsis* spp. infection of SS93-6012 may have been greater.

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