Planting Date and Cultivar Effects on Soybean Yield, Seed Quality, and *Phomopsis* sp. Seed Infection

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ABSTRACT

Wrather, J. A., Sleper, D. A., Stevens, W. E., Shannon, J. G., and Wilson, R. F. 2003. Planting date and cultivar effects on soybean yield, seed quality, and *Phomopsis* sp. seed infection. Plant Dis. 87:529-532.

Incidence of *Phomopsis* seed decay is frequently high and quality low in seed from early-maturing maturity group III and IV soybean cultivars planted in early to mid-April in the southern United States. Cultivars resistant to this disease have not been available until the recent release of germ plasm lines SS 93-6012 and SS 93-6181. Our objective was to determine the effects of planting dates with these lines and one *Phomopsis* seed decay-susceptible soybean cultivar, Asgrow 3834, on seed infection by *Phomopsis* spp. and on yield and the correlation between percentage of Asgrow 3834 infected with *Phomopsis* spp. and seed quality. Generally, yields averaged over years were significantly greater for mid-April than mid-June plantings, and yields of cultivars were similar within a planting date. Soybean lines SS 93-6012 and SS 93-6181 were highly resistant to *Phomopsis* seed decay compared with the susceptible cultivar, Asgrow 3834. There was a significant, negative correlation between germination of seed from mid-April plantings of Asgrow 3834 and percentage of these seed infected with *Phomopsis* spp. Moreover, there were significant correlations between fatty acid composition of Asgrow 3834 seed and the percentage of these seed infected with *Phomopsis* spp. This altered composition of fatty acids may be responsible for reduced quality of oil derived from seed infected with this fungus. *Phomopsis* seed decay-resistant soybean lines SS 93-6012 and SS 93-6181 should be useful in breeding programs focused on developing high-yielding cultivars resistant to this disease.

Additional keywords: fatty acid composition, *Glycine max*, percent oil, percent protein, seed germination

In the southern United States, drought stress has been a common yield-limiting factor for soybean, *Glycine max* (L.) Merr., especially if drought occurred during bloom and pod fill. In this region, maturity group (MG) V and VI soybean cultivars commonly are planted from mid-May to mid-June, and the plants flower and produce seed from late July to early September, when droughts most often develop. The Early Soybean Production System (ESPS) was designed to help growers in the south avoid late-July through early-September drought damage to soybean (9). The success of this system is dependent on planting early-maturing cultivars (cultivars

from 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. Advantages of the ESPS have been observed in Arkansas (21), Mississippi (9), Texas (17), and Missouri (24). Unfortunately, seed infection with *Phomopsis* spp. was high in some MG III and IV cultivars planted during mid-April in Arkansas (14) and Missouri (24).

Phomopsis seed decay is endemic throughout most soybean-growing states in the United States (20), but yield losses from this disease vary among years and among regions (25). *Phomopsis* seed decay suppressed yield, reduced the quality of seed used for planting by reducing germination, and lowered the quality of seed used for processing (19,24). The quality of oil derived from seed infected with *Phomopsis* spp. was lower than that from healthy seed (10), but the effects of *Phomopsis* seed decay on fatty acid composition of seed has not been determined.

The usefulness of the ESPS would be greater if *Phomopsis* spp.-resistant soybean cultivars were available for planting (14,24). Fortunately, a *Phomopsis* seed decay-resistant soybean line, MO/PSD-

0259, was registered in 1993 (15). Two high-yielding soybean lines, SS 93-6012 and SS 93-6181, resistant to *Phomopsis* seed decay were developed from a cross of MO/PSD-0259 and Asgrow 3322. The objective of this research was to determine the effects of planting dates of soybean lines SS 93-6012 and SS 93-6181 and one *Phomopsis* seed decay-susceptible cultivar, Asgrow 3834, on infection of seed by *Phomopsis* spp. and on yield and the correlation between percentage of Asgrow 3834 seed infected with *Phomopsis* spp. and seed quality.

MATERIALS AND METHODS

Experiment plot management. Plots were established in 1998, 1999, and 2000 near Portageville, MO on a silt loam soil consisting of 34% sand, 50% silt, and 16% clay. 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 was pushed off just prior to planting to form a flat-top ridge. Soybean cv. Asgrow 3834 (MG III) and soybean lines SS 93-6012 (MG III) and SS 93-6181 (MG IV) were planted, respectively, on 15 April, 15 May, and 15 June 1998; on 16 April, 17 May, and 14 June 1999; and 21 April, 16 May, and 15 June 2000. Each four-row plot was 3 m wide and 7.6 m long, and 26 seed were planted per meter of row. All plots received a pre-emergence application of imazaquin (American Cyanamid Co., Agriculture Division, Wayne, NJ) at 0.14 kg a.i./ha and alaclor (Monsanto Co., Agriculture Group, St. Louis, MO) at 0.4 kg a.i./ha. Plots were cultivated once and hand-weeded 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, and these areas were planted to soybean prior to this test. Plots were not irrigated. A split-plot design with four replications included three planting dates as main plots and soybean cultivars randomized within the main plots as subplots.

Seed analysis. Each year, the middle two rows of each plot were harvested at maturity, and seed moisture was measured. Yield of each plot was adjusted to 13% moisture. 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. (1998 to 2000) and seed qual-

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This research was, in part, supported by the Missouri Agricultural Experiment Station.

This project also was supported by the Missouri Soybean Farmer Checkoff through the Missouri Soybean Merchandising Council grant no. 99-185.

Accepted for publication 6 December 2002.

Publication no. D-2003-0226-01R
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ity traits (1999 to 2000). In all, 100 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) potato dextrose agar in petri dishes (22). 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 (2,20). During 1999 and 2000, germination of seed from all plots was determined. Germination of 100 arbitrarily chosen seed from each plot was tested according to procedures adopted by the Association of Official Seed Analysts (6). During 1999 and 2000, 20 g of seed from the Asgrow 3834 plot planted in mid-April were tested for protein and oil by near-infrared analysis. Seed from these same plots also were tested for fatty acid composition following the method described by Rebetzke et al. (16).

Analysis of data. Statistical analyses of treatment effects on seed yield, percentage of seed infected with *Phomopsis* spp., and percent germination 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 (18). Probability levels greater than 0.05 were categorized as nonsignificant. Data from 1998 and 2000 were used in the analysis of effects of planting date and cultivar on percent seed infected with *Phomopsis* spp. because few seed harvested from plots in 1999 were infected with this pathogen, except those from Asgrow 3834 planted in mid-April. Percent seed infected with *Phomopsis* spp. during 1998 and 2000 were transformed before analysis, $(X + 0.5)^{1/2}$, where *X* is the original percent infection (18), because the percentage of seed from all plantings of lines SS 93-6012 and SS 93-6181 infected with *Phomopsis* spp. was very low (<10%) during both years. The response variables percentage of oil and protein in seed, seed germination, and content of palmitic, stearic, oleic, linoleic, and linolenic in seed from mid-April-planted Asgrow 3834 were regressed against the explanatory variable percentage of seed infected with *Phomopsis* spp. (7). The PROC CORR procedure of the SAS software was used for this.

RESULTS

Yields averaged over planting dates and cultivars were greater in 2000 and less in 1998 and 1999 (2.37, 0.96, and 0.53 Mg ha⁻¹, respectively). Differences in yields

among years were due to differences in rainfall for June through August each year. Rainfall for these months during 1998, 1999, and 2000 were 22.8 cm, 15.0 cm, and 42.4 cm, respectively. Plant populations averaged over treatments, 205,000 plants ha⁻¹ 4 weeks after emergence, were similar among years (*data not shown*) and were not responsible for yield differences among years.

Analysis of variance for yield, percent *Phomopsis* sp. seed infection, and germination of harvested seed indicated that there was a significant planting date–cultivar interaction (Table 1). Generally, yields averaged over years were significantly greater for mid-April than mid-June plantings, and yields of cultivars and lines were similar within a planting date (Table 2). The percentage of Asgrow 3834 seed infected with *Phomopsis* spp. was significantly greater than for lines SS 93-6012 and SS 93-6181 seed for all planting dates (Table 2). The percentage of Asgrow 3834 seed infected with *Phomopsis* spp. was significantly greater for the mid-April than mid-May and significantly greater for the mid-May than mid-June plantings. Generally, germination of seed from mid-June plantings was significantly greater than seed from mid-April plantings and significantly greater for lines SS 93-6012 and SS 93-6181 than for Asgrow 3834 planted mid-April and mid-May (Table 2). Germination of seed from mid-June plantings for all cultivars was similar.

Analysis of variance for percent *Phomopsis* sp. seed infection and for germination of seed indicated that there was a significant year–cultivar interaction (Table 1). The percentage of Asgrow 3834 and line SS 93-6012 seed infected with *Phomopsis* spp. was significantly greater for 2000 than 1998 (Table 3). The percentage of Asgrow 3834 seed infected with *Phomopsis* spp. was significantly greater than lines SS 93-6012 and SS 93-6181 for 1998 and 2000. Germination of Asgrow 3834 seed was significantly greater for 1999 than 2000, and germination of lines SS 93-6181 and SS 93-6012 seed was significantly greater than Asgrow 3834 for 1999 and 2000 (Table 3).

Analysis of variance for yield, percent *Phomopsis* sp. seed infection, and germination of seed indicated that there was a significant year–planting date interaction (Table 1). Yields were significantly greater for all planting dates for 2000 than for 1998 and 1999 (Table 4). Yields were significantly greater for the mid-April than mid-May plantings in 2000 but not in 1998 and 1999. The percentage of seed infected with *Phomopsis* spp. was significantly greater for the mid-April and mid-May plantings for 2000 than for 1998 (Table 4). The percentage of seed infected with *Phomopsis* spp. was significantly greater for the mid-April than mid-May planting in 2000 and for the mid-April than mid-June

Table 1. Analysis of variance for yield, percent *Phomopsis* sp. infection, and percent seed germination for year, cultivar, and planting date^z

Source of variation	<i>Pr</i> ≥ <i>F</i>		
	Yield	Seed infected (%)	Germination (%)
Year (Y)	<0.0001	0.0244	0.0187
Planting date (PD)	0.0019	<0.0001	<0.0001
Y × PD	0.0002	0.0017	0.0059
Cultivar (C)	0.3356	<0.0001	<0.0001
Y × C	0.4055	0.0418	<0.0001
PD × C	0.0338	<0.0001	<0.0001
Y × PD × C	0.3916	0.6983	0.5110

^z Years for yield were 1998, 1999, and 2000. Years for percent seed infected with *Phomopsis* spp. were 1998 and 2000. Years for percent seed germination were 1999 and 2000. Cultivars were Asgrow 3834, SS93-6012, and SS93-6181. Planting dates were mid-April, mid-May, and mid-June.

Table 2. Planting date and soybean cultivar effects on yields (Mg ha⁻¹), percent *Phomopsis* sp. infection of seed, and germination of harvested seed averaged over years^z

Cultivars	Planting dates		
	mid-April	mid-May	mid-June
Yields (Mg ha ⁻¹)			
Asgrow 3834	1.43 ab	1.26 bcd	1.15 cd
SS 93-6012	1.29 bc	1.32 bc	1.17 cd
SS 93-6181	1.54 a	1.32 bc	1.12 d
Seed infection (%)			
Asgrow 3834	41.5 a	32.4 b	8.1 c
SS 93-6012	7.1 c	2.3 def	1.2 f
SS 93-6181	3.4 de	3.6 d	1.5 ef
Germination			
Asgrow 3834	39.1 e	56.0 d	88.9 ab
SS 93-6012	67.5 c	80.9 b	94.4 a
SS 93-6181	84.5 ab	88.6 ab	93.1 a

^z Years were 1998 to 2000 for effects on yields, 1998 and 2000 for effects on percent *Phomopsis* sp. seed infection, and 1999 and 2000 for effects on germination of harvested seed. Values followed by the same letter are not significantly different (*P* = 0.05).

planting in 1998 and 2000. Germination of seed from the mid-April planting was significantly greater for 1999 than 2000 and significantly greater for the mid-June than mid-April and mid-May plantings in 1999 and 2000 (Table 4).

There were no significant correlations between the percentage of seed infected with *Phomopsis* spp. and the percentage of oil and protein in seed from mid-April-planted Asgrow 3834 (Table 5). There was a significant negative correlation between the percentage of seed infected with *Phomopsis* spp. and germination of seed from mid-April plantings of Asgrow 3834. There was a significant and negative correlation between the percentage of seed infected with *Phomopsis* spp. and palmitic acid and oleic acid composition. There was a significant and positive correlation between the percentage of seed infected with *Phomopsis* spp. and linoleic acid and linolenic acid composition. There was no correlation between the percentage of seed infected with *Phomopsis* spp. and stearic acid composition of seed.

DISCUSSION

In this study, similar yields were observed for MG III and IV cultivars between mid-April and mid-May plantings in 2 of 3 years (1998 and 1999) in this nonirrigated field. These were dry years. Yields were significantly greater for mid-April than mid-May plantings in 1 of 3 years (2000). Rainfall for June through August was greater for 2000 (42.4 cm) than 1998 (22.8 cm) and 1999 (15.0 cm). Yield advantages for mid-April compared with mid-May plantings may be more evident in years when rain is abundant or in irrigated fields in Missouri. Yield advantages for mid- to late April compared with mid- to late May plantings of soybean have been reported from Texas (4) and Mississippi (8). However, Wrather et al. (24) determined that there were no differences in yields for soybean cultivars in MG IV, V, and VI between mid-April and mid-May plantings over 3 years in a nonirrigated Missouri field. There may be some benefits of early planting, including earlier maturity and, consequently, earlier harvest dates. Harvest then may be spread out over time for more efficient use of labor and machinery.

Seed harvested from early- to mid-April plantings of MG III and IV cultivars in Missouri and regions further south may not be suitable for planting due to low germination (8,14). Seed of these cultivars planted in April develop and mature during the hot summer months, and infection by *Phomopsis* spp. can be a problem (13,14,22,24). However, low germination may be due to factors other than *Phomopsis* spp. infection of seed. Our results also indicated that germination of *Phomopsis* spp.-resistant soybean lines that were planted in mid-April and matured during the hot summer was low. Mayhew and

Caviness (14) determined that germination of seed from *Phomopsis* spp.-resistant soybean line PI 417479 planted in mid-April was very low (50% in 1989 and 1990). Keigley and Mullen (12) concluded that low germination of seed from plants exposed to high day and night temperatures from R5 to maturity was associated with heat stress and not infection with *Phomopsis* spp.

We observed no correlation between percent oil or percent protein in seed and percentage of these seed infected with *Phomopsis* spp. Hepperly and Sinclair (10) observed significantly greater percent oil and percent protein in seed with symptoms of *Phomopsis sojiae* infection. We have no explanation for differences between their data and ours. Bradley et al. (5) reported a significant relationship between incidence of seed infected with *Phomopsis* spp. and

concentration of protein or oil within soybean seed at Champaign, IL in 1999 but not 1998 and not at Urbana, IL in 1998 or 1999. They did observe greater percent harvested seed infected with *Phomopsis* spp. in most cultivars at Champaign in 1999 than in 1998. They suggested that the severity of infection, which is influenced by environment, could play a role in determining how seed protein and oil are affected by infection by *Phomopsis* spp.

This is the first report of significant correlation between percent harvested seed infected with *Phomopsis* spp. and fatty acid composition of seed. This altered composition of fatty acids may be responsible for reduced quality of oil derived from seed infected with *Phomopsis* spp. Hepperly and Sinclair (10) reported that oil from seed with symptoms of *P. sojiae* infection was visibly darker and had

Table 3. Year and soybean cultivar effects on percent *Phomopsis* sp. infection of seed and germination of harvested seed averaged over planting dates^z

Year	Cultivars		
	Asgrow 3834	SS 93-6012	SS 93-6181
Seed infection (%)			
1998	20.3 b	1.8 d	2.5 d
2000	30.2 a	4.7 c	3.1 cd
Germination			
1999	75.3 c	84.8 ab	89.1 a
2000	47.3 d	77.1 bc	88.4 a

^z Planting dates were 15 April, 15 May, and 15 June 1998; 16 April, 17 May, and 14 June 1999; and 21 April, 16 May, and 15 June 2000. Percent *Phomopsis* sp. infection of seed data were collected for 1998, 1999, and 2000, but infection was extremely low for all plots in 1999 and these data were not included in this analysis. Values followed by the same letter are not significantly different ($P = 0.05$).

Table 4. Year and planting date effects on yields (Mg ha⁻¹), percent *Phomopsis* sp. infection of seed, and germination of harvested seed averaged over cultivars^z

Year	Planting date		
	mid-April	mid-May	mid-June
Yields (Mg ha ⁻¹)			
1998	0.95 de	0.93 de	0.99 d
1999	0.57 ef	0.49 f	0.53 f
2000	2.75 a	2.47 b	1.89 c
Seed infection (%)			
1998	8.8 bc	7.4 c	3.3 d
2000	19.1 a	11.6 b	2.7 d
Germination			
1999	77.2 b	77.3 b	94.7 a
2000	50.3 c	73.0 b	89.6 a

^z Cultivars were Asgrow 3834, line SS 93-6012, and line SS 93-6181. Percent seed infected with *Phomopsis* sp. was extremely low for all treatments in 1999, and data were excluded from the analysis. Values followed by the same letter are not significantly different ($P = 0.05$).

Table 5. Pearson correlation coefficients between quality parameters for Asgrow 3834 seed harvested from mid-April planting and percentage of these seed infected with *Phomopsis* spp.^z

Seed quality parameter	Pearson correlation coefficient	Probability
Percent oil	0.4208	0.2991
Percent protein	-0.0914	0.8295
Percent germination	-0.8903	0.0030
Palmitic acid composition	-0.7564	0.0298
Stearic acid composition	-0.0667	0.8752
Oleic acid composition	-0.8306	0.0106
Linoleic acid composition	0.8839	0.0036
Linolenic acid composition	0.7879	0.0202

^z $N = 8$.

an unpleasant, rancid odor compared with oil from seed without symptoms. They believed that damage to seed coat tissue (fissuring) due to *Phomopsis* sp. infection may expose the underlying tissue to air and consequent oxidation of lipids which does occur when soybean seed are dehulled (1). We determined that linolenic acid levels increased as percentage of seed infected with *Phomopsis* spp. increased, and high levels of this fatty acid are considered responsible for poor flavor and undesirable odors in soybean oil (3). Declines in palmitic and oleic acid levels due to infection of seed by *Phomopsis* spp. may influence the human health benefits of oil from these seed. Palmitic acid declined as percentage of seed infected with *Phomopsis* spp. increased and lower levels of this fatty acid are beneficial to humans. It has been shown to increase LDL levels, which are associated with increased heart disease (11). The lower levels of oleic acid are not desirable because increased levels of this monosaturated fatty acid may be beneficial for human nutrition (23).

This is the first report of successful management of *Phomopsis* seed decay in MG III and IV soybean cultivars planted in April in the south. This was accomplished by planting soybean lines SS 93-6012 and SS 93-6181, which are resistant to infection by this pathogen. All plots in our experiment were harvested when mature. The effects of delayed harvest on the percentage of lines SS 93-6012 and SS 93-6181 infected with *Phomopsis* spp. have not been determined. However, these lines yield well and will be useful in breeding programs focused on developing high yielding *Phomopsis* spp.-resistant cultivars.

ACKNOWLEDGMENTS

We thank J. Elrod for her efforts in this project.

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