

Soybean Seed Composition Constituents as Affected by Drought and *Phomopsis* in *Phomopsis* Susceptible and Resistant Genotypes

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*The objective of this research was to evaluate the effect of drought and *Phomopsis* on seed composition constituents in *Phomopsis* susceptible (S), moderately resistant (MR), and resistant (R) soybean genotypes grown under irrigated and non-irrigated environments. Genotypes of maturity group (MG) III and V were grown under field conditions in 2003 and 2005. Seed protein, oil, fatty acids, sugars, and minerals were evaluated in seeds harvested at harvest maturity (R8) and 15 days after harvest maturity (delayed harvesting). The results showed that seed protein and oleic acid were higher in S than in MR or R genotypes at 15 days after harvest maturity in MG III in non-irrigated soybean. For MG V genotypes, seed protein, oil, and oleic acid were higher and linoleic and linolenic acids were lower in MR and R than in S in irrigated*

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and non-irrigated soybean at harvest maturity and 15 days after harvest maturity. In MG III genotypes, seed sucrose, raffinose, and stachyose were higher in MR and R than in S genotypes in irrigated soybean at maturity only. In irrigated or non-irrigated soybean, seed sucrose, raffinose, and stachyose were higher in R than in MR or in S in MG V in 2003 and 2005 at harvest maturity or 15 days after harvest maturity. Seed minerals were also altered in MG III and V. This research demonstrated that seed composition components were altered by drought and Phomopsis and the degree of alteration depended on the level of resistance of the genotype and MG.

KEYWORDS Carbon isotope, nitrogen isotope, seed composition, seed oil, seed protein, seed sugars

INTRODUCTION

Soybean is a major crop in the world, and soybean seed is an important source for protein and oil (Belewu & Belewu 2007) and other nutrients including sugars and mineral nutrients (Hou et al. 2009; Zobiolo et al. 2010; Bellaloui et al. 2010, 2011). Soybean protein ranges from 341 to 568 g kg⁻¹ of total seed weight, with a mean of 421 g kg⁻¹. Oil ranges from 83 to 279 g kg⁻¹, with a mean of 195 g kg⁻¹ (Wilson 2004). Major saturated fatty acids include palmitic (10%–12%) and stearic (2.2%–7.2%; Cherry et al. 1985); major unsaturated fatty acids include oleic (24%), linoleic (54%), and linolenic (8%; Schnebly & Fehr 1993). Seed sugars include sucrose (4%–5%), raffinose (2%), and stachyose (3.5%–4.5%; Wilson, Novitzky, & Fenner 1995). Seed macro- and micro-nutrients concentrations for soybean were previously reported (Zobiolo et al. 2010; Bellaloui et al. 2010, 2011).

The early soybean production system showed yield benefit in Arkansas (Taylor 1999), Mississippi (Heatherly 1999), Texas (Savoy, Cothran, & Shumway 1992), and Missouri (Wrather et al. 1996). However, declines in seed quality under the early soybean production system conditions due to phomopsis seed decay, which is primarily caused by *Phomopsis longicolla* Hobbs (Mengistu et al. 2010; Wrather et al. 1996) and heat (Smith et al. 2008), have been a major concern. Smith et al. (2008) reported that early maturing soybean cultivars mature in the early soybean production system between mid-August and mid-September under high temperatures and high relative humidity. These conditions are favorable for the development of *P. longicolla*, leading to poor seed germination and poor vigor (TeKrony et al. 1996; Mengistu & Heatherly 2006; Smith et al. 2008) and a decrease in oil quality (Hepperly & Sinclair 1978).

Currently, there are no known phomopsis seed decay resistant soybean cultivars available in the market, but phomopsis seed decay resistant germplasm has been developed. For example, germplasm genotypes MO/PSD- 0259 (Minor et al. 1993), SS 93-6012, and SS 93-6181 (Pathan et al. 2009) were shown to be resistant to *Phomopsis sp.* in Missouri. Seed composition has been shown to be affected by phomopsis seed decay. For example, for soybean genotypes SS 93-6012, SS 93-6181, and Asgrow 3834, grown across a range of planting dates (mid-April, mid-May, and mid-June), a significant negative correlation between the percentage of seed infection and palmitic and oleic acids, and significant positive correlation between seed infection and linoleic acid and linolenic acids were found (Wrather et al. 2003). However, no significant correlations between the percentage of seed infection and the percentage of oil, protein, or stearic acid in seed were found (Wrather et al. 2003). Other researchers observed significantly higher oil and protein percentages in seed with symptoms of *Phomopsis sojae* infection (Hepperly & Sinclair 1978). Bradley et al. (2002) reported a low but significant positive correlation between incidence of seed infected with *Phomopsis spp.* and concentration of seed protein or oil at Champaign, IL, in 1999, but not at Urbana, IL, in 1998 or 1999. This inconsistency reflects the fact that the *Phomopsis spp.* infection level was higher in Champaign in 1999 than in Urbana in 1998 and 1999. The severity of *Phomopsis spp.* infection on seed quality is highly dependent on the environment, especially moisture (Mengistu et al. 2007; Smith et al. 2008). It was also reported that linolenic acid concentration increased as percentage of infested seed increased (Bradley et al. 2002), which is undesirable for oil quality because this fatty acid when degraded (oxidized) is responsible for poor flavor and undesirable odors in soybean oil (Beare-Rogers 1995). The possible explanation of the negative effect of *Phomopsis* on oil as suggested by Hepperly & Sinclair (1978) was that the darker and rancid odor oil from seed with symptoms of *Phomopsis sojae* infection compared with oil from seed without symptoms could be due to the damage to seed coat tissue and the exposure of the underlying tissue to air and consequent oxidation of lipids.

It was found that oil, meal, and flour derived from infected seeds had lower quality than those from non-infected seeds (Roy 1976), and this could be due to the degradation of seed coat proteins by fungal released enzymes (Ryley 2004). Fungus infected soybean seed showed higher protein concentrations, lower carbohydrates, and no change or increased oil concentrations (Pathan, Sinclair, & McClary 1989). Wilson, Novitzky, and Fenner (1995) found a positive correlation between fungal damage and both protein and oil concentrations (increase in oil from 19.5% to 22.8%, and protein from 43.7% to 50.8%). The positive correlation was explained as a consequence of residual seed mass loss.

Since the early soybean production system conditions are favorable for the development of *P. longicolla* and since very limited information

is available on the effect of drought and *Phomopsis* on seed composition in soybean genotype differing in susceptibility to *Phomopsis*, the current research was conducted. Therefore, the objective of this research was to investigate the effect of drought and *Phomopsis* on seed protein, oil, fatty acids, sugars, and mineral levels in genotypes differing in their susceptibility to *P. longicolla*. Since mineral nutrition is essential for protein and oil metabolism and can be affected by biotic and abiotic stress environments, macro- and micro-nutrients were also investigated.

MATERIALS AND METHODS

Two field experiments were conducted in 2003 and 2005 at the Delta Research and Extension Center, Stoneville, MS (33° 26' N, 90° 91'W). The soil was a Sharkey clay soil (very-fine, smectitic, thermic, Chromic Epiaquert). One experiment was overhead irrigated and the other experiment was not irrigated. The irrigated and non-irrigated plots were separated by a buffer of 15 rows (9.9 m) to prevent irrigation drift. The detailed description of the irrigation, design, and equipment used in this experiment were previously reported elsewhere (Mengistu et al. 2010). Within each irrigation environment, genotypes were grouped by MG. Harvest times (maturity harvest at R8 and 15 days after maturity harvest) were treatments nested within genotypes and MG. Genotypes were planted in a randomized complete block design with three replications. Irrigation and MG were not replicated. The same genotypes and experimental location were used each year.

Planting dates were 12 May 2003 and 10 May 2005. Soybean genotypes of MG III and MG V were planted in single-row plots 2.7 m long, 0.66 m wide, and at a seeding rate of 75 seed per row. Susceptible (S), moderately resistant (MR), and resistant (R) genotypes were used and classified as indicated by Mengistu et al. (2010). The MG III genotypes were Cumberland and Fremont (susceptible), PI 594401A and Williams 82 (moderately resistant), and PI 594618B and PI 594778 (resistant). For MG V, the genotypes were 95-231-3BF and Freedom (susceptible), D86-4565 and PI 200510 (moderately resistant), and Forrest and NCPR83-45 (resistant). Seeds were harvested at harvest maturity (R8) (Fehr & Caviness 1977) and 15 days after harvest maturity (delayed harvesting). Harvesting dates for MG III genotypes under the non-irrigated conditions were 30 July in 2003 and 5 August in 2005. For irrigated conditions, harvesting dates were 10 August in 2003 and 15 August in 2005. Harvesting dates for MG V genotypes under the non-irrigated conditions were 1 October in 2003 and 3 October in 2005. For irrigated conditions harvesting dates were 5 October in 2003 and 30 September in 2005. For the irrigated treatment, soil water potential was kept between 0 and -17 kPa until harvest. For non-irrigated treatment, the range of soil water potentials ranged from -76 to -184 kPa.

Inoculation and Disease Rating

Inoculation and disease ratings were described in Mengistu et al. (2010). A conidial suspension (10⁶ conidia mL⁻¹) was sprayed onto the plants. Plants were sprayed at dusk. Since the R3 growth stage was different in each MG category, plastic shields were placed between plots to prevent spore drift.

Disease ratings were made according to Mengistu et al. (2010), and percent seed infection index (PSII) was used for each genotype for measuring resistance. The PSII was calculated by dividing the percent seed infection for each genotype averaged across replicates by the percent seed infection of the most susceptible accession (standard) selected within early and late MG multiplied by 100. The PSII was proved to be more consistent than percent infection seed (Mengistu et al. 2007; Mengistu al. 2010). Genotypes were classified as resistant = 1% to 10%; moderately resistant = 11% to 20%; moderately susceptible = 21% to 30%; and susceptible >30% (Mengistu et al. 2010).

Sample Selection

Phomopsis evaluation and data analysis from this experiment were presented in Mengistu et al. (2010). Here we selected a subset of MG III (6 out of 14 genotypes) and MG V (6 out of 9 genotypes) from those trials and analyzed the seed composition. The selection of the subset was based on the consistency of these genotypes to fit the three selected levels of *Phomopsis* resistance (susceptible [S], moderately resistant [MR], and resistant [R]). The MG III genotypes were Cumberland and Fremont (susceptible), PI 594401A and Williams 82 (moderately resistant), and PI 594618B and PI 594778 (resistant). For MG V, the genotypes were 95-231-3BF and Freedom (susceptible), D86-4565 and PI 200510 (moderately resistant), and Forrest and NCP83-45 (resistant).

Seed Analysis for Protein, Oil, and Fatty Acids

Mature seed at harvest maturity and after 15 days after harvest maturity were analyzed for protein, oil, and fatty acids. About 25 g of seed from each plot was ground using a Laboratory Mill 3600 (Perten, Springfield, IL). Analyses were conducted by near infrared reflectance (Wilcox & Shibbles 2001) using a diode array feed analyzer AD 7200 (Perten, Springfield, IL). Calibrations were developed by the University of Minnesota, using Perten's Thermo Galactic Grams PLS IQ software. The calibration curve was established according to AOAC methods (1990a, 1990b). Analyses of protein and oil were performed based on a seed dry matter basis (Wilcox & Shibbles 2001; Boydak et al. 2002). Fatty acids were analyzed on an oil basis.

Seed Analysis for Sucrose, Raffinose, and Stachyose

Mature seed at R8 and 15 days after R8 were analyzed for sucrose, raffinose, and stachyose concentrations. About 25 g of seed from each plot were ground using a Laboratory Mill 3600 (Perten, Springfield, IL). Analyses were conducted by near infrared reflectance (NIR) (Wilcox & Shibbles 2001; Bellaloui et al. 2009) using an AD 7200 array feed analyzer (Perten, Springfield, IL). Analyses of sugars were performed based on a seed dry matter basis (Wilcox & Shibbles 2001; Boydak et al. 2002).

Seed N, S, and Mineral Composition

Mature seed at harvest maturity and 15 days after harvest maturity were collected and ground to pass through a 1-mm sieve using a Laboratory Mill 3600 (Perten, Springfield, IL). Seed N, S, and mineral concentrations were analyzed at the University of Georgia's Soil, Plant, and Water Laboratory in Athens, GA. Seed K, Ca, Zn, Mn, and Cu concentrations were analyzed by digesting 0.5 g of dried ground seed in HNO₃ in a microwave digestion system. Values were then determined using inductively coupled plasma spectrometry. Nitrogen and S were measured in a 0.25-g sample using an elemental analyzer (LECO CNS-2000, LECO Corporation, MI). Seed P, B, and Fe concentrations were determined as indicated in the following sections.

Boron Measurement

Boron concentration was measured in mature seed at harvest maturity and 15 days after harvest maturity from each replicate and each treatment with the Azomethine-H method (Lohse 1982). Detailed description of the method was previously reported elsewhere (Dordas 2006; Bellaloui et al. 2010). Briefly, a 1.0-g seed sample was ashed at 500 °C, and then extracted with 20 ml of 2 M HCl at 90°C for 10 min and filtered. The filtered mixture was transferred to plastic vials. A 2-ml sample of the solution was added to 4 ml of buffer solution (containing 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid) and 4 ml of freshly prepared azomethine-H solution (0.45% azomethine-H and 1% of ascorbic acid) (John, Chuah, & Neufeld 1975). Boron concentration was measured in samples at room temperature after at least 45 min for color development. Boron concentration was determined using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA) at 420 nm.

Iron Measurement

Iron concentration was measured in mature seed at harvest maturity and 15 days after harvest maturity. Seed Fe was measured after

acid-wet digestion, extraction, and reaction of reduced ferrous Fe with 1, 10-phenanthroline (Bandemer & Schaible, 1994; Loeppert & Inskeep, 1996; Bellaloui et al. 2010). Briefly, a 2-g ground seed sample was digested in nitric acid (70% m/m HNO₃). After the acids were removed by volatilization, the soluble constituents were dissolved in 2 M HCl. Standard solutions of iron were prepared in 0.4 M HCl and ranged from 0.0 to 4 $\mu\text{g ml}^{-1}$ Fe. Phenanthroline solution of 0.25% m/v was prepared in 25% v/v ethanol. A fresh quinol solution (1% m/v) reagent was prepared on the day of use. A 4-ml aliquot was pipetted into 25-ml volumetric flask. A concentration of 0.4 M HCl solution was used to dilute the aliquot to 5 ml. A volume of quinol solution was added and mixed. Then, 3 ml of phenanthroline solution and 5 ml of tri-sodium citrate solution (8% m/v) were added. The mixture containing the aliquot, HCl, phenanthroline, and tri-sodium citrate was diluted to 25 ml. The mixture stood for 4 h, and the absorbance of the samples was read at 510 nm using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA).

Phosphorus Measurement

Phosphorus concentration was measured in seed at harvest maturity (R8) and 15 days after harvest maturity. Phosphorus concentration was measured spectrophotometrically as the yellow phospho-vanado-molybdate complex (Analytical Methods Committee 1959; Bellaloui et al. 2010). Briefly, 2 g of dry, ground seed were ashed and then 10 ml of 6 M HCl was added. The samples were placed in a water bath at 70°C to evaporate and dry. After drying, the samples were kept under heat, and 2 ml of 36% m/m HCl was added and gently boiled. Then, 10 ml of water was added and the solution was carefully boiled for about 1 min. The samples were transferred and diluted to 50 ml in a volumetric flask. After the first 2 ml were discarded, the sample solution was then filtered and kept for P analysis. A 5-ml sample was taken, and 5 ml of 5 M HCl and 5 ml of ammonium molybdate-ammonium metavanadate (a solution of ammonium molybdate, (NH₄)₂MoO₄ (25 g/500 ml water), and ammonium metavanadate, NH₄VO₃) (1.25 g/500 ml water) reagent were added, diluted to 50 ml. A concentration ranging from 0–50 $\mu\text{g/ml}$ phosphorus was prepared for standard solution using dihydrogen orthophosphate dissolved in both water and 36% m/m HCl. To measure P concentrations, samples were allowed to stand for 30 min at ambient temperature before the samples were read at 400 nm using a Beckman Coulter DU 800 spectrophotometer at 400 nm (Fullerton, CA).

Data Analysis

Analysis of variance was performed as previously described by Mengistu et al. (2010) using Proc Mixed Model procedure in SAS (SAS Institute 2001),

TABLE 1 Analysis of variance (F value and level of significance) of seed composition components as affected by harvesting time (at harvest maturity or 15 days after harvest maturity), genotype, year, and their interactions in maturity group III soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*). The experiment was grown under irrigated environment in 2003 and 2005 at Jamie Whitten Delta States Research Center, Stoneville, MS

Source of variability	Organic compounds							
	Protein	Oil	Oleic	Linoleic	Linolenic	Sucrose	Raffinose	Stachyose
Harvesting	NS	NS	6.48**	7.75**	7.75**	NS	21***	12***
Year	46***	NS	NS	NS	NS	NS	214***	228***
Genotype	2.91*	20***	NS	27***	6.38***	34***	34***	19***
Harvesting × Year	58***	NS	5.23*	43***	NS	NS	21***	12***
Harvesting × Genotype	7.21***	2.6*	NS	NS	3.78**	3.69***	5.26***	2.45*
Year × Genotype	4.45**	5.64***	44***	15***	3.15*	15.11***	2.97*	NS
Harvesting × Year × Genotype	29***	5.09***	3.69**	16***	21***	3.69**	5.26***	2.45*

Source of variability	Non-organic compounds							
	N	Ca	K	B	Mn	Cu	Zn	
Harvesting	NS	6.64**	NS	26***	38***	NS	38***	
Year	NS	16***	192***	48**	178***	230***	76***	
Genotype	21***	13***	18***	17***	41***	16***	53***	
Harvesting × Year	33***	NS	5.92*	81***	20***	5.74*	453***	
Harvesting × Genotype	NS	NS	NS	NS	NS	3.05*	NS	
Year × Genotype	NS	NS	4.46**	NS	NS	NS	NS	
Harvesting × Year × Genotype	NS	NS	NS	NS	NS	3.91**	NS	

*, **, and *** significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

and means were separated using Fisher's least significant difference test at $P \leq 0.05$. Since irrigation and maturity treatments were not replicated (Mengistu et al. 2010) and since year and genotype interacted for some seed composition components (Tables 1 through 4), we presented the results by each irrigation × MG × year treatment combination.

RESULTS

Analysis of Variance

For MG III under the irrigated environment, analysis of variance showed that the main effect of harvesting time, year, and genotype were significant for some seed composition component variables (Table 1). The interaction effects between harvesting time, year, and genotype for seed composition constituents were different (Table 1) depending on the sensitivity of each

TABLE 2 Analysis of variance (F value and level of significance) of seed composition components as affected by harvesting time (at harvest maturity or 15 days after harvest maturity), genotype, year, and their interactions in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*). The experiment was grown under irrigated environment in 2003 and 2005 at Jamie Whitten Delta States Research Center, Stoneville, MS

Source of variability	Organic compounds							
	Protein	Oil	Oleic	Linoleic	Linolenic	Sucrose	Raffinose	Stachyose
Harvesting	NS	NS	6.64**	11***	10**	NS	NS	NS
Year	59**	NS	8.54*	6.46**	NS	10*	270***	124***
Genotype	92***	46***	37***	20***	NS	154***	49***	37***
Harvesting × Year	NS	NS	15***	NS	NS	NS	NS	NS
Harvesting × Genotype	NS	NS	2.84*	NS	NS	NS	NS	NS
Year × Genotype	4.03**	NS	2.52*	NS	NS	14***	6.98***	2.64*
Harvesting × Year × Genotype	2.61*	NS	NS	NS	NS	NS	NS	NS

Source of variability	Non-organic compounds							
	N	Ca	K	B	Mn	Cu	Zn	
Harvesting		24***	9.25***	0.8	45***	NS	NS	192***
Year		NS	18*	121***	161***	155***	121***	226***
Genotype		19***	23***	32***	36***	69***	18***	49***
Harvesting × Year		NS	NS	NS	NS	14***	NS	NS
Harvesting × Genotype		NS	NS	NS	NS	NS	NS	NS
Year × Genotype		NS	NS	2.6*	NS	NS	3.55**	NS
Harvesting × Year × Genotype		NS	NS	NS	NS	NS	NS	NS

*, **, and *** significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

genotype to the growing conditions in each year and harvesting time. A similar observation was noticed on the effect of the main factors and their interactions on seed composition components in MG V, although the type of the affected component was not always the same (Table 2). For example, in some cases the effect of the main factors and their interactions was similar between MG III and MG V such as in case of the effect of harvesting time on protein, oil, oleic and linolenic acids, Ca, B, Cu, and Zn (Table 1 and Table 2). In other cases, the effect of the factors and their interactions on seed composition components was different between MG III and MG V such as in case of the effect of year on oleic and linoleic acids and sucrose, or the effect of year × genotype on protein, oil, and oleic acid (Table 1 and Table 2). A similar observation was noticed for MG III and V under the non-irrigated environment (Table 3 and Table 4), although the pattern was different between MG III and MG V. The different effects of these factors on seed composition component indicate the significant

TABLE 3 Analysis of variance (F value and level of significance) of seed composition components as affected by harvesting time (at harvest maturity or 15 days after harvest maturity), genotype, year, and their interactions in maturity group III soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*). The experiment was grown under non-irrigated environment in 2003 and 2005 at Jamie Whitten Delta States Research Center, Stoneville, MS

Source of variability	Organic compounds							
	Protein	Oil	Oleic	Linoleic	Linolenic	Sucrose	Raffinose	Stachyose
Harvesting	NS	NS	NS	27***	8.57**	9.98**	51***	21***
Year	21***	34***	NS	NS	NS	27**	44***	22***
Genotype	24***	35***	53***	NS	NS	78***	92***	45***
Harvesting × Year	5.91*	12***	23***	9.72**	3.88*	9.98**	59***	21***
Harvesting × Genotype	NS	NS	6***	NS	NS	NS	NS	NS
Year × Genotype	NS	NS	NS	3.52**	NS	16***	2.36*	4.81***
Harvesting × Year × Genotype	NS	NS	NS	16***	17***	NS	NS	NS

Source of variability	Non-organic compounds							
	N	Ca	K	B	Mn	Cu	Zn	
Harvesting		66***	NS	NS	107***	9.5**	9**	483***
Year		NS	22**	62***	435***	508***	222***	686***
Genotype		29***	18***	11**	21***	44***	11***	51***
Harvesting × Year		NS	NS	NS	12***	17***	4.91*	43***
Harvesting × Genotype		NS	NS	NS	NS	NS	3*	NS
Year × Genotype		NS	3.2*	4.37**	NS	NS	NS	NS
Harvesting × Year × Genotype		NS	NS	NS	NS	NS	2.49*	NS

*, **, and *** significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

effect of growing conditions in each year on the genotype and harvesting time, and suggest that different responses of seed composition components exist between genotypes depending on harvesting time and growing season (Tables 1–4).

Concentrations of Seed Protein, Oil, Fatty Acids, Sugars, and Minerals

For GM III under the irrigated environment, oleic acid concentration was consistently higher in MR and R genotype in 2003 and 2005 at both harvest maturity and 15 days after harvest maturity (Table 5 and Table 6). No consistency in protein, oil, linoleic, and linolenic acid concentrations was observed (Table 5 and Table 6). Sucrose concentration was higher in R genotypes; raffinose concentration was higher in MR and R lines; and stachyose concentration was higher in MR and R genotypes at harvest maturity only, in

TABLE 4 Analysis of variance (F value and level of significance) of seed composition components as affected by harvesting time (at harvest maturity or 15 days after harvest maturity), genotype, year, and their interactions in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*). The experiment was grown under non-irrigated environment in 2003 and 2005 at Jamie Whitten Delta States Research Center, Stoneville, MS

Source of variability	Organic compounds							
	Protein	Oil	Oleic	Linoleic	Linolenic	Sucrose	Raffinose	Stachyose
Harvesting	NS	NS	NS	28***	16***	7.06*	11**	NS
Year	NS	4.65*	13***	NS	NS	52***	NS	NS
Genotype	78***	33***	71***	32***	16***	123***	62***	26***
Harvesting × Year	6.61**	9.91**	23***	NS	NS	8.64**	233***	80***
Harvesting × Genotype	NS	2.55*	4.76***	2.51*	5.42***	11	NS	NS
Year × Genotype	5.68***	NS	NS	NS	NS	3.71**	NS	3.26**
Harvesting × Year × Genotype	NS	5.22***	NS	NS	NS	7.34***	NS	NS

Source of variability	Non-organic compounds						
	N	Ca	K	B	Mn	Cu	Zn
Harvesting	20***	NS	31***	274***	326***	99***	713***
Year	NS	21**	50***	74***	80***	60***	150***
Genotype	19***	14***	25***	19***	90***	16***	44***
Harvesting × Year	NS	NS	17***	NS	7.97**	29***	6.02*
Harvesting × Genotype	NS	NS	NS	NS	3.72**	NS	NS
Year × Genotype	NS	NS	NS	NS	NS	NS	NS
Harvesting × Year × Genotype	NS	NS	NS	NS	NS	NS	NS

*, **, and *** significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

addition to the higher stachyose in R genotypes at 15 days after harvest maturity as well (Table 5 and Table 6). Mineral nutrition concentration was also affected by the level of susceptibility of genotypes. For example, K, Mn, and Zn were higher in MR and R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity, and at 15 days after harvest maturity only for Cu concentration (Table 5 and Table 6)

Under the non-irrigated environment in MG III, protein and oleic acid were higher at harvest maturity and 15 days after harvest maturity in S genotypes than in MR or R genotypes. Oil was higher in MR or R genotypes than in S genotypes. This pattern was shown in 2003 and 2005 (Table 7 and Table 8). No consistency was shown in linoleic or linolenic acids. Sucrose concentration was higher in R genotypes than in S or MR genotypes at harvest maturity and 15 days after harvest maturity. Raffinose and stachyose concentrations were higher in MR and R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity (Table 7 and Table 8).

TABLE 5 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group III soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under irrigated environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2003.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	390	410	183	173	293	284	527	563
Fremont (S)	403	410	192	193	285	291	513	563
PI 594401A (MR)	436	350	213	210	223	233	567	467
Williams 82 (MR)	387	373	220	213	237	223	580	450
PI 594618B (R)	421	383	225	233	223	223	583	477
PI 594778 (R)	428	393	225	230	223	237	583	463
LSD	8.0	10.1	5.4	7.1	10.3	8.5	12.4	20.2

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	47	97	38	38	6.0	5.7	24	24
Fremont (S)	53	107	33	33	4.0	4.0	26	26
PI 594401A (MR)	83	57	42	42	10.0	9.7	35	35
Williams 82 (MR)	70	67	43	43	12.0	11.7	36	36
PI 594618B (R)	80	67	55	55	12.0	11.7	35	35
PI 594778 (R)	80	60	56	56	12.0	11.7	35	35
LSD	5.7	6.4	2.1	2.0	0.9	0.9	0.8	0.8

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	5.8	5.3	1.1	1.1	0.2	0.23	26	22	14	15	4.0	3.4	33	24
Fremont (S)	5.4	5.0	1.1	1.0	0.2	0.26	23	23	15	13	3.7	3.7	33	25
PI 594401A (MR)	5.0	3.7	1.3	1.2	0.3	0.37	36	29	22	23	6.3	5.9	45	37
Williams 82 (MR)	4.6	3.8	1.2	1.2	0.4	0.40	34	29	23	24	6.7	6.1	44	34
PI 594618B (R)	4.1	3.3	1.4	1.3	0.3	0.36	35	33	22	25	6.0	5.8	41	32
PI 594778 (R)	4.1	3.3	1.4	1.3	0.3	0.34	37	34	23	25	6.0	6.5	41	32
LSD	0.3	0.2	0.06	0.05	0.009	0.03	1.1	2.3	1.6	0.9	0.6	0.53	1.1	1.1

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

Seed K, Mn, and Zn concentrations were higher in MR and R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity (Table 7 and Table 8). Boron and Cu concentrations were higher in MR and R genotypes at harvest maturity only. Calcium concentration was higher in R genotypes at harvest maturity only (Table 7 and Table 8). Nitrogen had the opposite trend in that N concentration was higher in S genotypes than in MR or R genotypes (Table 7 and Table 8).

TABLE 6 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group III soybean genotypes differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under irrigated environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2005.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	434	377	185	220	181	237	628	583
Fremont (S)	443	417	186	217	181	197	585	563
PI 594401A (MR)	394	430	211	217	261	290	432	457
Williams 82 (MR)	407	457	226	223	235	290	425	467
PI 594618B (R)	381	473	221	213	284	257	422	507
PI 594778 (R)	389	447	225	200	285	280	418	483
LSD	6.6	6.1	6.0	5.8	13.6	8.0	18.0	19.2

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	100	93	42	48	15	17	43	38
Fremont (S)	98	80	44	47	15	17	44	44
PI 594401A (MR)	63	87	37	44	23	18	56	47
Williams 82 (MR)	65	73	36	41	23	17	61	44
PI 594618B (R)	63	80	57	43	23	17	50	53
PI 594778 (R)	65	77	49	46	24	17	64	49
LSD	4.5	5.3	1.9	2.1	1.2	1.0	3.6	3.6

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
	Cumberland (S)	4.7	5.7	1.5	1.4	0.2	0.2	31	35	20	25	6.6	7.3	28
Fremont (S)	5.1	5.1	1.3	1.4	0.3	0.3	27	38	21	24	10.2	7.3	26	44
PI 594401A (MR)	3.8	4.6	1.8	2.0	0.3	0.3	31	48	29	33	9.9	10.7	39	56
Williams 82 (MR)	3.7	4.2	1.9	2.0	0.3	0.4	33	51	27	35	7.6	11.7	36	54
PI 594618B (R)	3.5	4.2	1.6	1.9	0.3	0.3	32	47	24	33	7.7	10.3	37	55
PI 594778 (R)	3.5	4.4	1.7	1.9	0.4	0.4	34	48	26	34	10.3	11.3	38	53
LSD	0.2	0.3	0.10	0.8	0.03	0.02	2.8	2.5	1.7	1.04	0.5	0.8	1.9	1.6

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

For MG V under the irrigated environment, concentrations of seed protein, oil, and oleic acid were consistently higher in MR and R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity in 2003 and 2005. There was lower linoleic acid at harvest maturity and 15 days after harvest maturity, and lower linolenic at 15 days after harvest maturity only (Table 9 and Table 10).

Seed raffinose and stachyose concentrations were higher in R and MR genotypes than in S genotypes at harvest maturity and 15 days after harvest

TABLE 7 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group III soybean genotypes differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under non-irrigated environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2003.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	410	426	171	157	293	278	527	549
Fremont (S)	422	426	180	173	285	283	513	578
PI 594401A (MR)	380	364	213	190	223	188	567	442
Williams 82 (MR)	387	357	210	197	237	188	580	435
PI 594618B (R)	390	363	217	213	223	178	583	452
PI 594778 (R)	403	377	207	229	223	201	542	449
LSD	7.2	8.9	6.6	8.5	10.3	7.8	19.7	21.7

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	47	102	38	33	5.7	10	23	29
Fremont (S)	53	108	33	29	4.0	9	26	34
PI 594401A (MR)	83	63	42	38	9.7	15	35	44
Williams 82 (MR)	70	72	43	38	11.7	16	36	42
PI 594618B (R)	80	73	55	51	11.7	17	35	43
PI 594778 (R)	80	73	56	51	11.7	16	35	43
LSD	5.7	7.8	2.0	2.0	0.9	1.0	0.8	0.9

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
	Cumberland (S)	5.8	5.0	1.1	1.0	0.2	0.2	26	12	14	10	4.0	2.0	33
Fremont (S)	5.3	4.7	1.1	1.0	0.2	0.2	23	14	15	8	4.0	2.6	33	17
PI 594401A (MR)	5.0	3.7	1.3	1.2	0.2	0.2	36	24	22	19	6.3	4.1	45	29
Williams 82 (MR)	4.5	3.8	1.2	1.1	0.3	0.3	34	22	23	18	6.7	4.7	44	27
PI 594618B (R)	4.4	3.3	1.4	1.3	0.3	0.3	35	23	22	21	6.0	4.7	41	24
PI 594778 (R)	4.2	3.4	1.3	1.3	0.3	0.3	37	24	23	19	6.0	5.0	41	24
LSD	0.3	0.2	0.07	0.05	0.01	0.02	1.0	1.4	1.6	1.2	0.6	0.7	1.1	0.9

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

maturity in 2003 and 2005 (Table 9 and Table 10). Seed concentration of sucrose was higher in R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity in 2003 and 2005. Seed sucrose in MR genotypes was not consistent. Seed concentrations of K, B, Mn, Cu, and Zn were higher in R and MR genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity (Table 9 and Table 10). Seed Ca concentration was higher in R and MR genotypes than in S lines at 15 days

TABLE 8 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group III soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under non-irrigated environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2005.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	437	443	176	199	287	320	533	437
Fremont (S)	457	444	185	186	263	330	559	443
PI 594401A (MR)	376	397	213	230	235	207	515	547
Williams 82 (MR)	403	395	227	233	206	190	507	550
PI 594618B (R)	367	405	220	236	233	230	497	503
PI 594778 (R)	407	404	220	237	197	240	513	543
LSD	13.9	9.0	6.4	6.3	13.4	13.1	30.0	17.4

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	MH	DH	MH	DH	MH	DH	MH	DH
Cumberland (S)	90	63	47	47	10	10	36	36
Fremont (S)	80	60	49	49	10	10	35	35
PI 594401A (MR)	73	93	42	42	18	18	49	49
Williams 82 (MR)	77	90	41	41	18	18	52	52
PI 594618B (R)	73	93	62	62	17	17	43	43
PI 594778 (R)	72	80	54	54	19	18	55	55
LSD	5.9	6.1	1.7	1.7	0.7	0.8	3.3	3.3

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
Cumberland (S)	5.5	4.8	1.6	1.5	0.2	0.2	39	40	25	26	8.0	8.0	45	36
Fremont (S)	5.9	5.1	1.4	1.3	0.3	0.3	39	37	24	26	7.7	11	44	35
PI 594401A (MR)	4.6	3.9	1.8	1.8	0.3	0.3	52	40	35	35	10.7	11	55	47
Williams 82 (MR)	4.4	3.7	2.0	1.9	0.3	0.3	51	43	33	32	11.3	9.0	54	44
PI 594618B (R)	4.3	3.6	1.7	1.6	0.3	0.4	49	42	30	32	12.0	9.0	53	45
PI 594778 (R)	4.3	3.6	1.7	1.7	0.4	0.4	52	44	31	31	12.0	11.6	56	46
LSD	0.24	0.2	0.10	0.10	0.02	0.03	2.7	2.7	0.8	1.4	1.06	0.5	1.4	1.9

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

after harvest maturity only. Seed N concentration was higher in S genotypes than in R or MR genotypes at harvest maturity and 15 days after harvest maturity consistently in 2003 and 2005 (Table 9 and Table 10). Except for Ca, similar pattern of seed composition components was repeated for MG V under the non-irrigated environment (Table 11 and Table 12). Seed Ca was not consistent over years.

TABLE 9 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under irrigated (I) environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2003.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	377	373	177	180	217	227	583	577
Freedom (S)	413	383	188	177	197	223	563	537
D86-4565 (MR)	430	443	217	240	290	313	457	460
PI 200510 (MR)	457	457	228	230	290	317	467	463
Forrest (R)	473	473	241	230	257	330	507	440
NCPR83-45 (R)	447	470	241	230	280	303	483	430
LSD	5.0	7.6	8.0	5.3	7.3	11.2	19.3	16.2

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	93	90	48	48	8.7	8.7	34	34
Freedom (S)	80	97	47	47	8.3	8.3	37	37
D86-4565 (MR)	87	63	44	44	18	18	47	47
PI 200510 (MR)	73	63	41	41	17	17	44	44
Forrest (R)	80	60	62	62	17	17	53	53
NCPR83-45 (R)	77	60	67	67	17	17	49	49
LSD	5.3	6.6	1.4	1.3	0.9	0.9	2.7	2.7

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	5.7	4.9	1.4	1.4	0.2	0.2	35	32	25	22	7	6.7	41	32
Freedom (S)	5.6	5.0	1.3	1.4	0.3	0.3	38	32	24	24	7	6.3	44	35
D86-4565 (MR)	4.6	3.8	2.0	2.0	0.3	0.4	48	44	33	30	11	8.4	56	46
PI 200510 (MR)	4.2	3.5	2.0	2.0	0.4	0.4	51	42	35	34	12	12.1	54	45
Forrest (R)	4.2	3.5	1.9	1.9	0.3	0.4	47	41	33	34	10	12.0	55	45
NCPR83-45 (R)	4.6	4.4	1.9	1.9	0.4	0.4	48	43	34	32	11	11.0	53	44
LSD	0.3	0.2	0.08	0.1	0.02	0.04	2.5	1.7	1.04	1.0	0.8	0.8	1.6	1.7

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

DISCUSSION

Seed Protein, Oil, and Fatty Acids in MG III and MG V

The higher oleic acid concentrations in MR and R genotypes than in S genotypes at harvest maturity or 15 days after harvest maturity in MG III under the irrigated environment may be due to the ability of M and R genotypes to accumulate higher level of oleic acid than in S genotypes, or may be due *phomopsis* susceptibility differences. Previous research showed

TABLE 10 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under irrigated (I) environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2005.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	366	378	183	190	217	213	582	597
Freedom (S)	381	382	187	187	204	230	587	583
D86-4565 (MR)	416	425	228	233	283	291	547	467
PI 200510 (MR)	428	422	234	220	293	252	507	480
Forrest (R)	433	430	212	233	243	255	518	440
NCPR83-45 (R)	437	428	224	230	281	245	497	453
LSD	6.1	7.6	8.4	5.1	15.9	11.6	33.3	19.0

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	99	97	36	36	5.3	5.3	24	25
Freedom (S)	101	93	35	35	4.7	4.7	26	25
D86-4565 (MR)	67	67	43	43	8.0	8.0	39	39
PI 200510 (MR)	74	60	45	45	10.0	10.0	39	40
Forrest (R)	73	63	61	65	10.3	10.3	39	36
NCPR83-45 (R)	64	57	63	67	8.7	8.7	38	37
LSD	5.4	6.8	2.9	1.4	0.9	0.9	1.8	0.7

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	6.0	5.2	1.0	1.0	0.2	0.2	26	23	14	14	4.3	4.3	33	24
Freedom (S)	5.8	5.0	1.1	1.1	0.2	0.2	25	24	14	14	3.7	4.4	34	25
D86-4565 (MR)	4.1	3.3	1.3	1.4	0.2	0.3	37	25	20	23	7.3	4.7	45	36
PI 200510 (MR)	4.4	4.1	1.4	1.5	0.3	0.4	38	32	19	25	5.3	6.1	42	34
Forrest (R)	4.4	3.7	1.5	1.5	0.3	0.3	40	34	24	26	6.3	5.6	44	34
NCPR83-45 (R)	4.6	3.8	1.4	1.4	0.3	0.3	39	36	23	23	6.7	6.5	42	33
LSD	0.33	0.3	0.07	0.07	0.007	0.007	1.3	1.8	1.7	0.9	0.8	0.6	1.1	1.7

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

that susceptible genotypes to charcoal rot disease pressure resulted in higher oleic acid concentrations (Bellaloui, Mengistu, & Paris 2008). The inconsistency of protein, oil, linoleic, and linolenic acids concentration in these lines suggests the significant influence of environmental factors such as temperature and rain fall in each year on these components (Maestri et al. 1998; Piper & Boot 1999; Dardenelli et al. 2006; Bellaloui, Mengistu, & Paris 2008; Bellaloui et al. 2009). The ability to maintain these compounds at higher levels in M and R genotypes than in S genotypes may depend on MG since this

TABLE 11 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under non-irrigated (NI) environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2003.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)							
	HM	DH	HM	DH	HM	DH	HM	DH						
95-231-3BF (S)	377	366	169	194	237	191	583	562						
Freedom (S)	413	386	187	180	197	178	563	512						
D86-4565 (MR)	430	423	199	224	290	278	457	445						
PI 200510 (MR)	457	443	228	210	290	271	467	438						
Forrest (R)	473	460	225	210	257	295	507	425						
NCPR83-45 (R)	447	442	248	210	280	258	483	405						
LSD	5.0	6.8	8.2	7.4	8.0	11.4	19.2	16.2						
Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)							
	HM	DH	HM	DH	HM	DH	HM	DH						
95-231-3BF (S)	93	90	48	43	8.7	13	34	42						
Freedom (S)	80	93	47	43	8.3	13	37	46						
D86-4565 (MR)	87	67	44	39	18	22	47	53						
PI 200510 (MR)	73	68	41	37	17	22	44	52						
Forrest (R)	80	60	62	57	17	21	53	59						
NCPR83-45 (R)	77	60	63	63	17	22	49	57						
LSD	5.3	4.2	2.0	1.4	0.9	1.10	2.7	2.8						
Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	5.7	5.1	1.4	1.3	0.2	0.2	35	25	25	17	7.3	7.7	41	24
Freedom (S)	5.1	4.8	1.4	1.3	0.3	0.3	38	25	24	20	7.3	5.5	44	27
D86-4565 (MR)	4.2	3.8	2.0	1.9	0.3	0.4	48	35	33	25	11	7.0	56	39
PI 200510 (MR)	4.2	3.4	2.0	2.0	0.4	0.3	51	31	35	30	12	11	54	37
Forrest (R)	4.2	3.4	1.9	1.8	0.3	0.3	47	31	33	28	10	11	55	38
NCPR83-45 (R)	4.4	3.7	1.9	1.8	0.4	0.4	48	32	34	28	11	7.8	53	36
LSD	0.3	0.2	0.08	0.08	0.02	0.03	2.5	2.0	1.04	1.4	0.8	0.6	1.6	1.8

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

observation was noticed in MG V and was not in MG III. Alternatively, the different responses between MG III and MG V cultivars could be related to the different environmental conditions experienced during seed maturation. The MG III cultivars would have been exposed to hotter and perhaps wetter conditions.

The lower protein and oil concentrations shown in S genotypes in MG V under the irrigated environment may be due to weak cell wall or seed coat integrity compared with MR and R genotypes. Weak cell wall

TABLE 12 Effect of harvest maturity (HM) or 15 days after harvest maturity (delayed harvesting [DH]) on seed protein, oil, fatty acids, sugars, and mineral nutrition concentrations in maturity group V soybean genotypes that differ in their susceptibility to *Phomopsis* (*Phomopsis longicolla*) under non-irrigated (NI) environment at Jamie Whitten Delta States Research Center, Stoneville, MS, in 2005.¹

Genotype	Protein (g/kg)		Oil (g/kg)		Oleic (g/kg)		Linoleic (g/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	351	369	171	167	197	177	555	582
Freedom (S)	389	374	176	169	151	185	561	558
D86-4565 (MR)	444	436	198	220	261	293	535	441
PI 200510 (MR)	426	448	209	234	235	284	481	465
Forrest (R)	421	427	204	230	251	286	482	415
NCPR83-45 (R)	439	434	202	224	242	277	488	438
LSD	12.9	6.9	6.1	7.2	7.1	12.8	18.3	19.0

Genotype	Linolenic (g/kg)		Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)	
	HM	DH	HM	DH	HM	DH	HM	DH
95-231-3BF (S)	88	102	42	31	15	11	43	32
Freedom (S)	92	94	44	31	15	10	44	34
D86-4565 (MR)	87	73	37	39	23	13	56	48
PI 200510 (MR)	83	65	36	41	23	15	61	47
Forrest (R)	78	70	54	61	23	16	50	46
NCPR83-45 (R)	83	62	49	62	24	14	64	44
LSD	4.6	5.3	1.8	1.6	1.2	1.1	3.6	1.0

Genotype	N (%)		K (%)		Ca (%)		B (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)	
	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	HM	DH	MH	DH
95-231-3BF (S)	5.4	5.2	1.5	1.0	0.2	0.2	30	14	19	8.3	6.6	2.9	37	16
Freedom (S)	5.9	5.0	1.3	1.1	0.3	0.2	30	14	19	9.1	6.4	3.2	36	17
D86-4565 (MR)	4.6	3.3	1.8	1.3	0.3	0.2	43	16	29	18	9.3	3.5	47	27
PI 200510 (MR)	4.4	4.7	1.9	1.4	0.3	0.3	42	23	28	19	10.1	4.7	45	26
Forrest (R)	4.2	3.7	1.6	1.4	0.3	0.3	39	25	24	21	10.6	4.4	45	26
NCPR83-45 (R)	4.2	3.8	1.7	1.3	0.3	0.3	42	27	26	17	10.8	5.1	47	25
LSD	0.2	0.2	0.10	0.07	0.03	0.007	2.7	2.0	1.01	0.9	1.07	0.6	1.5	1.7

¹Means were separated using Fisher's least significant difference test at $P \leq 0.05$.

or lack of seed coat integrity may lead to loss of soluble compound from seed or lead to pathogen penetration through the seed coat, resulting in breakdown of organic compounds, including protein and oils. Therefore, the level of resistance to *Phomopsis* and seed coat composition and integrity may determine the leakage rate and loss of soluble compounds (Marshner 1995; Spann & Schumann 2011). Soybean seed protein and oil are rich in essential amino acids and fatty acids, which are favorable conditions for fungal pathogens to grow and multiply (Welbaum 2006), leading to chemical

breakdown of protein, oil, and fatty acids (Begum et al. 2008). Our results showed that MG III genotypes did not follow the same trend as those of MG V genotypes for protein, oil, and fatty acids, and this may be due to genotype differences (Bellaloui & Mengistu 2008), maturity differences (Bellaloui et al. 2009), or exposure to different environmental conditions during seed maturation.

Literature on the effect of *Phomopsis* on seed composition is limited, and what is available is on susceptible genotypes to *Phomopsis* and not on genotypes differing in their susceptibility to *Phomopsis* as in the current study. Previous research showed conflicting results regarding the relationship between *Phomopsis* and seed composition components. It was found that there were no significant correlations between the percentage of seed infection and seed protein and oil in soybean cultivar Asgrow3834 planted in mid-April (Wrather et al. 2003). However, a significant negative correlation between the percentage of seed infection and seed palmitic and oleic acids, and a significant positive correlation between seed infection and linoleic and linolenic acids, was observed (Wrather et al. 2003). Other researchers found higher oil and protein percentages in seed with symptoms of *Phomopsis sojae* infection (Hepperly & Sinclair 1978); a positive correlation between soybean seed protein concentration and fungal damage by *Fusarium* spp., *Cercospora* spp., and *Phomopsis* spp. (Wilson, Novitzky, & Fenner 1995); and higher seed protein in seed inoculated with *Colletotrichum truncatum* than those in the un-inoculated seeds (Begum et al. 2008).

Fungal-infected soybean seed had higher protein concentrations (Meriles et al. 2004), lower carbohydrates, and no change or increased oil concentrations (Pathan, Sinclair, & McClary 1989.). Fungal infection by *C. truncatum* increased protein and oleic acid content and reduced linoleic acid content but did not change in extracted oil and other fatty acids when compared with un-inoculated seeds after four days of incubation (Begum et al. 2008). *C. truncatum* infection did not change the amount of extracted oil in inoculated seeds compared to un-inoculated seeds, but increased oleic acid and decreased linoleic acid (Bhattacharya & Raha 2002). The increase in protein and oleic acid was also reported in corn, groundnut, and soybean seeds infected by different fungal species, including *Aspergillus*, *Penicillium*, *Fusarium*, *Curvularia*, *Alternaria*, and *Rhizopus* (Bhattacharya & Raha 2002). Meriles et al. (2004) also detected a high level of oleic acid and lower levels of linoleic and linolenic acids in *Fusarium* spp. and *Diaporthe/Phomopsis* complex-infected soybean seeds. The increase of protein and oleic acid in S genotypes in MG V at harvest maturity and 15 days after harvest maturity in 2003 and 2005 under the non-irrigated environment in our results is supported by previous research (Wilson, Novitzky, & Fenner 1995; Begum et al. 2008; Meriles et al. 2004). However, our results in MG III or MG V under the irrigated

environment cannot be compared to the literature as the available literature is on the effect of *Phomopsis* on seed composition in inoculated and non-inoculated susceptible genotypes. To our knowledge this is the first detailed report that investigated the effect of *Phomopsis* on seed composition components in genotypes differing in their susceptibility to *Phomopsis* infection.

Mechanisms of the effect of *Phomopsis* on seed protein, oil, and saturated and unsaturated fatty acids are still not yet understood, but it was suggested that the fungal damage could be due to the disruption of cellular membranes, probably causing hydrolysis of hydratable phosphatides, principally lecithins by phospholipase D activity (List, Mounts, & Lanser 1992). The other possible explanation is that *Phomopsis* may inhibit the uptake and assimilation of essential macro- and micronutrients involved in seed coat resistance against diseases, including *Phomopsis*. Minerals are involved in membrane permeability and integrity (Ca and B), phenol metabolism, and lignin biosynthesis (B, Mn, and Cu; Marschner 1995). Altering the balance of these nutrients in seed coat integrity, cell wall, and cell membrane, and changing the cell membrane phospholipids as a result of altered fatty acids, may undermine the integrity of cell wall and cell membrane to prevent disease from penetration (Marschner 1995).

Seed Sucrose, Raffinose, and Stachyose

The consistent higher levels of raffinose and stachyose in MR and R genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity in 2003 and 2005 under the irrigated and non-irrigated environment indicates that *Phomopsis* seed infection altered seed sugars, and there is an indirect possible role of specific sugars in *Phomopsis* resistance. The inconsistency of seed sucrose concentration in MR may indicate that the level of sucrose in genotypes may depend on the level of susceptibility of this genotype to *Phomopsis*. To maintain higher soybean seed sucrose concentration under *Phomopsis* infection, resistant soybean cultivars to *Phomopsis* may be needed. The low effect of *Phomopsis* on raffinose and stachyose compared to sucrose suggests that raffinose and galactinol levels may play an important role under abiotic stress, and the accumulation of galactinol and raffinose may protect the plant from stress environment such as drought (Taji et al. 2002) or diseases. Previous research showed that the activity of sucrose synthase, the main enzyme involved in sucrose hydrolysis in nodules, decreased under conditions of drought (Streeter 2003), and a several-fold decline in sucrose synthase was observed in soybean (González et al. 1995), suggesting the higher sensitivity of sucrose to biotic and abiotic stress than raffinose and stachyose.

Seed Mineral Composition

The lower seed concentrations of K, B, Mn, Cu, and Zn in R and MR genotypes than in S genotypes at harvest maturity and 15 days after harvest maturity in 2003 and 2005 may indicate the indirect involvement of these nutrients in the *Phomopsis* infection. The higher Ca concentration in MR and R genotypes than in S genotypes at 15 days after harvest maturity only may indicate that delaying harvesting results in the losing of Ca through leakage. This was not obvious at mature harvesting because the weathering effect that resulted from delaying harvesting was not a factor. The higher seed N in S genotypes than in MR or R genotypes indicated that *Phomopsis* infection may have resulted in higher N concentration in S lines as a result of disease pressure. The possible explanation for the lower concentration of seed K, Ca, Mn, B, Zn, and Cu in S than in MR or R genotypes may be due to *Phomopsis* infection that resulted in inhibition of the uptake and translocation of these nutrients (Spann & Schumann 2011) to the cotyledons. Previous research indicated that, although resistance is genetically controlled, environmental factors such as mineral nutrition can play an important role in resistance or tolerance to pathogens (Marschner 1995; Spann & Schumann 2011). Marschner (1995) reported that, as a rule, the effect of mineral nutrition is relatively small in highly resistant or highly susceptible cultivars, but it is substantial in moderately susceptible or resistant cultivars. The higher concentrations of these minerals in MR and R genotypes indicate that these minerals may be associated with *Phomopsis* resistance. It was also reported that phenolics and lignin are the key in defense mechanism to plants, and B, Mn, and Cu play a major role in phenolics and lignin synthesis (Marschner 1995; Graham 1983; Graham & Webb 1991). The role of minerals in cell wall and membrane integrity was also previously reported. For example, potassium (Spann & Schumann 2011) and Zn (Bolle-Jones & Hilton 1956) deficiency resulted in high cell wall leakage of sugar and amino acid concentrations to leaf apoplast, leading to easy penetration by pathogens. Boron deficiency resulted in higher fungal infection (Schutte 1967) and low content of Ca in plant tissue, increasing cell wall leakage of sugars and amino acids from cytoplasm to apoplast. It was found that Ca is involved in the stability of cell wall through polygalacturonates, which are required for the middle lamella for cell wall synthesis. The activity of enzymes like polygalacturonase, which dissolve the middle lamella, is inhibited drastically by Ca. Copper was found to inhibit diseases, and Cu deficiency resulted in impairment of defense compound production, accumulation of soluble carbohydrates, and reduced lignification, leading to lower disease resistance (Spann & Schumann 2011). The inconsistency of seed minerals in MG III genotypes may be due to genotype differences and maturity compared with MG V genotypes. Our results with MG V genotypes support previous research in that seed K,

Ca, Mn, B, Zn, and Cu may play an important role in *Phomopsis* resistance, and delayed harvesting may negatively affect the integrity of seed coat, increasing leakage of soluble compounds (Marshner 1995; Spann & Schumann 2011).

CONCLUSIONS

Our research demonstrated that *Phomopsis* affected seed protein, oil, and sugars under irrigated and non-irrigated conditions. The effect of *Phomopsis* and irrigation on seed protein, oils, and sugars depended on the genotype and maturity. The higher levels of Ca, K, B, Mn, and Zn in seed of resistant genotypes suggest that these minerals may be associated with *Phomopsis* infection and drought stress.

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