Relationships Between Oleic and Linoleic Acid Content and Seed Colonization by *Cercospora kikuchii* and *Diaporthe phaseolorum*

H. Q. Xue, Crop Science Department, North Carolina State University, Raleigh 27695; R. G. Upchurch and P. Kwanyuen, United States Department of Agriculture–Agricultural Research Service, Soybean and Nitrogen Fixation Unit, Raleigh, NC 27695

ABSTRACT

Xue, H. Q., Upchurch, R. G., and Kwanyuen, P. 2008. Relationships between oleic and linoleic acid content and seed colonization by *Cercospora kikuchii* and *Diaporthe phaseolorum*. Plant Dis. 92:1038-1042.

Compared with standard cultivars, seed of mid-oleic soybean genotypes sometimes have shown increased colonization by *Cercospora kikuchii* in the field as judged by increased levels of purple-stained seed. To examine relationships between oleic and linoleic acid levels in soybean seed and postharvest seed colonization by two fungal seed pathogens, we inoculated seed with differing oleic:linoleic acid (O/L) ratios. Seed with defined O/L ratios were produced by allowing seed development of two isogenic soybean lines to occur in three different air temperature environments. Seed produced in these environments were harvested, individually analyzed for fatty acid composition, and inoculated with mycelium preparations of the fungal seed pathogens *C. kikuchii* or *Diaporthe phaseolorum* var. *sojae*. Fungal biomass of infected seed was quantified by measuring in vitro ergosterol content. For both soybean lines, colonization by *C. kikuchii* was positively correlated with the O/L ratio ($r = 0.55$, $P < 0.03$) and oleic acid content ($r = 0.61$, $P < 0.02$), and negatively correlated with linoleic ($r = -0.60$, $P < 0.02$) and linolenic ($r = -0.58$, $P < 0.03$) acid content. No association was found between the extent of seed colonization by *D. phaseolorum* and the seed O/L ratio. Our data suggest that the O/L ratio may be related to soybean seed colonization by *C. kikuchii*, but there is no evidence of a relationship with *D. phaseolorum* var. *sojae* colonization.

Soybean (*Glycine max* (L.) Merr.) genotypes with mid-oleic acid content in the seed have been developed as a response to the changing concerns and needs of industry, agriculture and the consumer. Genotypes that produce seed with mid-oleic acid content (50 to 60%, compared with the 20 to 25% seed oleic acid content in standard soybean cultivars) also have reduced linoleic and linolenic acid content and yield oil that is more nutritionally healthful and oxidatively stable (21). Anecdotal field observations suggest that some mid-oleic soybean genotypes may have increased susceptibility to fungal pathogens during late seed development compared with standard cultivars. *Cercospora kikuchii* (Matsumoto & Tomoy), the

causal agent of purple seed stain (11,12), and *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *sojae*, a causal agent of Phomopsis seed decay (16,18), are widespread and agriculturally important soybean seed pathogens. The level of seed colonization by these fungi can be an important factor for seed quality evaluation and has, in cases of severe infestation, negatively impacted the economic return to soybean farmers (17). Moderate to severe levels of infection can result in reductions in seed grade, decreased rates of seed germination, and lower quality meal and oil.

Although relatively little is known about the constituents of soybean seed that affect susceptibility to fungal pathogens, one report does suggest that seed proteins may contribute to the seed-*C. kikuchii* interaction. Experiments demonstrated that soybean seed proteins induced the production of the phytotoxin cercosporin, a virulence factor, and altered gene expression in this pathogen (7). More evidence, however, suggests that it is the lipid components of seed that substantially regulate the interaction of fungal pathogens with soybean seed. Experiments with soybean cotyledons demonstrated that the application of lipase from *Aspergillus flavus* Link resulted in the generation of the aldehyde hexanal by the seed lipoxigenase pathway, and fungal spore germination was inhibited (6). The addition of linoleic and lino-

lenic acids but not oleic acid to soybean homogenates also inhibited spore germination. Significant correlations were reported between the fatty acid composition of seed of soybean cv. Asgrow 3834 and the percentage of these seed infected with *Phomopsis* spp. in the field (23). Increased seed infection was significantly ($P < 0.05$) positively correlated with linoleic and linolenic acid, and negatively correlated with palmitic and oleic acid levels. Recently, in vitro ergosterol assays were used to quantify the colonization by *C. kikuchii* and *D. phaseolorum* of soybean seed from genotypes with different oleic and linoleic acid contents. *C. kikuchii* seed colonization was greater in the higher oleic and less in the higher linoleic genotype, whereas colonization by *D. phaseolorum* was significantly greater in the higher linoleic and lower in the higher oleic acid genotype (26). The latter experiment demonstrated that seed fatty acid composition could alter the soybean-fungal interaction but did not take into account the potential complicating effect that the differing genetic backgrounds of the soybean genotypes used could have on colonization.

To address this complication and to examine the intrinsic effect of seed fatty acid composition on one component of pathogen susceptibility, soybean seed colonization, we used temperature manipulation during seed development to produce isogenic seed with different oleic and linoleic contents. Fungal ergosterol measurements and a protocol similar to the one designed to measure the influence of the high-oleic trait on the ability of peanut to support postharvest production of aflatoxin by *A. flavus* (24,25) were used to evaluate the effect of differing oleic:linoleic acid (O/L) ratio on *C. kikuchii* and *D. phaseolorum* colonization of postharvest-inoculated soybean seed.

MATERIALS AND METHODS

Plant cultivars and plant growth. The two soybean lines N0304-303-3 and N0304-303-7 were provided by J. W. Burton, Agricultural Research Service Soybean & Nitrogen Fixation Unit, North Carolina State University. These are F4-derived lines from the third backcross of the mid-oleic line, N00-2989, to N97-3120. N97-3120 is a low-palmitic low-linolenic F5-derived line from N87-2117-3 × Brim (1). The oleic acid content of ma-

Corresponding author: R. G. Upchurch
E-mail: greg.upchurch@ars.usda.gov

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

Accepted for publication 19 February 2008.

doi:10.1094/PDIS-92-7-1038

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2008.

ture field-grown seed of the cultivars was N3034-303-3, 57.4% and N3034-303-7, 35.0%. These oleic acid levels are significantly higher than the usual 20 to 25% found in seed of standard cultivars (21). Seed were pregerminated and planted in 25-cm pots containing a standard growth mix and grown at the Southeastern Plant Environment Laboratory (Phytotron), North Carolina State University. Ten pots (two cultivars by five replications) were arranged in a randomized complete block design in each of three growth chambers. Pots were watered with deionized water twice daily and with Phytotron nutrient solution (13) on Monday, Wednesday, and Friday (MWF). From day 1 to 14, the chambers were set to a day and night (D/N) of 14/10 h and a temperature (T) of 26/22°C with 1 h of light interruption at night to synchronize plant development. From day 15 to 48, the chambers were set for D/N = 12/12 and T = 26/22°C with no dark interruption. From day 49 to seed maturity, chamber 1 was maintained at T = 26/22°C, D/N = 12/12; chamber 2 was switched to and maintained at T = 22/18°C, D/N = 12/12; and chamber 3 was T = 34/26°C, D/N = 12/12.

Fungal growth and inoculation. *C. kikuchii* ATCC 86864, a toxigenic strain, provided by J. B. Sinclair (University of Illinois, Urbana) and *D. phaseolorum* isolate DPM, obtained from the National Soybean Pathogen Collection Center, University of Illinois, Urbana were grown and maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit) using hyphal tip transfer. To produce liquid cultures, two 5-mm agar plugs were taken from the periphery of a 4-day-old fungal colony and homogenized by vortexing (10 min) in a sterile screw-top tube that contained 4 ml of sterile water and 2.5 cm of sterile 4-mm glass beads. Each suspension (1 ml) was inoculated into 50 ml of PD medium in a 125-ml flask. Liquid cultures were agitated (180 rpm) at 23°C in a lighted incubator-shaker. On day 4, the mycelial mass was fragmented in a blender by blending the entire fungal culture at top speed for 20 s, pausing for 10 s, then blending again for 20 s. The mycelial fragments were har-

vested by vacuum filtration, dried on the filter for 5 min, and weighed on an analytical balance. A standardized suspension for the inoculum was produced by adjusting mycelial weight to approximately 2 g per 100 ml in sterile water, followed by mixing. Cotyledons were separated and surface sterilized by immersion in a 0.5% solution of NaOCl for 5 min followed by three rinses in sterile water. The cotyledons then were placed on the top surface of a stack of three 2.5-cm discs of sterile P5 filter paper (Fisher Scientific, Pittsburgh). Cotyledons were inoculated with a 5- μ l drop of the mycelium suspension of the pathogen or mock inoculated with a 5- μ l drop of sterile water as a control. Single-inoculated or control cotyledons were enclosed in 3.5-cm-diameter sterile plastic petri dishes. Petri dishes were arranged on trays in a randomized complete block design. Each tray was enclosed in a plastic bag to maintain humidity and incubated for 7 days in a 23°C chamber with a cycle of 12 h of fluorescent light and 12 h of darkness. Experiment design for postharvest seed colonization studies with each pathogen was as follows. For each O/L ratio seed class, an experimental unit consisted of five cotyledons inoculated with the pathogen and five cotyledons mock inoculated with sterile water. This experimental unit was replicated three times in an experiment and the experiment was repeated. Thus, for each O/L ratio seed class inoculated with a single pathogen, a total of 60 observations was recorded. On day 7, cotyledons were lyophilized to obtain a dry weight measurement, then each ground to fine powder and stored separately frozen until extraction for ergosterol. Fungal growth was estimated by measuring ergosterol content as described by Xue et al. (26).

Analysis of seed fatty acid composition. Fatty acid methyl esters (FAMES) of seed chip samples were prepared for acid methanolysis (27). Approximately 25 mg of seed tissue was heated to and held at 85°C for 90 min in a 5% HCl-95% methanol solution. FAMES were partitioned 2 $\times$ into hexane and transferred to 2-ml vials for analysis. The FAMES were separated

by gas chromatography using an HP 6890 GC (Agilent Technologies, Inc., Wilmington, DE) equipped with a DB-23 30-m-by-0.53-mm column (same source). Operating conditions were 1- μ l injection volume, a 20:1 split ratio, and He carrier gas flow of 6 ml min⁻¹. Temperatures were 250, 200, and 275°C for the injector, oven, and flame ionization detector, respectively. Chromatograms were analyzed using HP ChemStation software (Agilent Technologies, Inc.). Fatty acid contents were calculated from chromatogram peak areas expressed as milligrams of fatty acid per gram of oil. Five seeds were chosen for each soybean line-temperature combination (experimental unit) based on the similarity of fatty acid profiles from chips taken from individual seed.

Ergosterol assay. Powdered inoculated and mock-inoculated cotyledon samples (approximately 100 mg) were extracted overnight at room temperature with 2 ml of chloroform:methanol (1:1). Ergosterol extraction was done in 1-dram brown glass, teflon screw-top sealed vials on a shaker (180 rpm) as described by Gilbert et al. (8). The top 500 μ l of the extract was removed from the vial, avoiding particulates, and passed through a 0.45- μ m syringe filter. Samples were stored at 4°C until analysis by high-performance liquid chromatography. Samples (20 μ l) were injected and analyzed using a Hewlett Packard 1050 automated sampling instrument. An isocratic program was run at room temperature using a mobile phase of 100% methanol at a flow rate of 1 ml/min. The 4.6-mm-by-25-cm C₁₈ column with guard was supplied by Alltech Associates, Inc (Deerfield, IL). Absorbance was monitored at 282 nm. Ergosterol eluted at about 10 min and was identified by comparison of the retention time with that of an authentic ergosterol standard supplied by Aldrich (Milwaukee, WI). A standard curve obtained by analyzing a series of ergosterol dilutions produced a highly significant linear relationship between ergosterol concentration and the peak area ($r^2 = 1$, $P < 0.001$). Calculations were done to express ergosterol as micrograms per gram of cotyledon dry weight.

Table 1. Fatty acid composition of mature seed of soybean lines N0304-303-3 and N0304-303-7 grown in three different air temperature environments during seed development

Soybean, environment (D/N, °C) ^y	Fatty acid, mean $\pm$ standard deviation (mg g ⁻¹ oil) ^x					O/L ^z
	Palmitate	Stearate	Oleate (O)	Linoleate (L)	Linolenate	
N0304-303-3						
22/18	44.3 $\pm$ 2.0 a	25.5 $\pm$ 0.7 a	251.1 $\pm$ 27.5 a	600.5 $\pm$ 22.7 a	78.6 $\pm$ 9.6 a	0.42 $\pm$ 0.1 a
26/22	45.2 $\pm$ 4.4 a	24.3 $\pm$ 1.7 ab	382.6 $\pm$ 36.6 b	488.4 $\pm$ 32.2 b	59.6 $\pm$ 6.7 b	0.79 $\pm$ 0.1 b
34/26	41.2 $\pm$ 2.8 b	24.3 $\pm$ 1.4 b	579.0 $\pm$ 55.9 c	324.0 $\pm$ 53.7 c	31.5 $\pm$ 3.0 c	1.86 $\pm$ 0.5 c
N0304-303-7						
22/18	42.2 $\pm$ 1.4 a	25.5 $\pm$ 1.6 a	256.7 $\pm$ 34.0 a	599.7 $\pm$ 31.4 a	76.0 $\pm$ 8.8 a	0.43 $\pm$ 0.1 a
26/22	40.6 $\pm$ 1.6 a	23.9 $\pm$ 0.5 b	335.5 $\pm$ 28.7 a	541.0 $\pm$ 24.3 b	58.9 $\pm$ 5.9 b	0.62 $\pm$ 0.1 a
34/26	38.2 $\pm$ 2.1 b	20.7 $\pm$ 1.3 c	364.5 $\pm$ 61.1 b	532.2 $\pm$ 58.1 b	44.4 $\pm$ 2.7 c	0.72 $\pm$ 0.2 b

^x Means followed by the same letter are not significantly different ($P < 0.05$) by t test.

^y D/N = day and night growth temperature.

^z O/L = soybean seed oleic:linoleic acid ratio.

Statistical analyses. Data were subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS (version 9.1; SAS Institute, Cary, NC). Means were separated by Fisher's protected *t* test using an α level of 0.05. Correlations between independent variables (seed fatty acids) and the fungal biomass marker ergosterol, the dependent variable, were calculated with the PROC CORR procedure in SAS.

RESULTS

Temperature manipulation to produce seed with different O/L ratios. Temperature manipulation during seed development produced three mature seed fatty acid types for each of the two soybean lines, N3034-303-3 and N3034-303-7 (Table 1). For both lines, increasing growth temperature (D/N = 22/18, 26/22, and 34/26°C) during pod fill significantly increased oleic acid ($P < 0.0001$) and the O/L ratio ($P < 0.0001$), and significantly

Table 2. Ergosterol content of cotyledons of soybean lines N3034-303-3 and N3034-303-7 differing in oleic and linoleic acid ratios, following postharvest inoculation with *Cercospora kikuchii* or *Diaporthe phaseolorum*

Pathogen, soybean line	D/N (°C) ^x	Mean ± standard deviation ^w	
		O/L ratio ^y	Ergosterol (µg/g) ^z
<i>C. kikuchii</i>			
N0304-303-3	22/18	0.42 ± 0.06 a	55.80 ± 9.49 a
	26/22	0.79 ± 0.13 b	76.43 ± 21.81 ab
	34/26	1.86 ± 0.46 c	90.92 ± 20.36 b
N0304-303-7	22/18	0.43 ± 0.08 a	30.15 ± 6.75 a
	26/22	0.62 ± 0.08 a	54.50 ± 14.59 ab
	34/26	0.72 ± 0.21 b	71.40 ± 40.18 b
<i>D. phaseolorum</i>			
N0304-303-3	22/18	0.42 ± 0.06 a	67.26 ± 45.00 a
	26/22	0.79 ± 0.13 b	161.69 ± 52.00 a
	34/26	1.86 ± 0.46 c	80.98 ± 59.46 a
N0304-303-7	22/18	0.43 ± 0.08 a	115.68 ± 69.00 a
	26/22	0.62 ± 0.08 a	77.65 ± 36.00 a
	34/26	0.72 ± 0.21 b	89.03 ± 57.09 a

^w Means followed by the same letter are not significantly different ($P > 0.05$) by *t* test.

^x D/N = day and night growth temperature.

^y O/L = soybean seed oleic:linoleic acid ratio.

^z Cotyledon dry weight. Means for ergosterol are based on 60 observations for each of the six O/L seed classes per pathogen.

Table 3. Relationships between the seed fatty acid composition of N3034-303-3 and N3034-303-7 and ergosterol content following inoculation with *Cercospora kikuchii*^z

Variable 1, variable 2	N3034-303-3		N3034-303-7	
	Corr.	P value	Corr.	P value
Ergosterol				
O/L	0.5374	0.0388	0.5656	0.0280
Palmitate	-0.1131	0.6882	-0.3551	0.1940
Stearate	-0.1289	0.6470	-0.6025	0.0175
Oleate	0.6174	0.0142	0.5986	0.0184
Linoleate	-0.6190	0.0139	-0.5708	0.0263
Linolenate	-0.6025	0.0174	-0.5518	0.0330
O/L				
Palmitate	-0.4435	0.0978	-0.7727	0.0007
Stearate	-0.1289	0.6470	-0.6009	0.0178
Oleate	0.9763	<0.0001	0.9861	<0.0001
Linoleate	-0.9755	<0.0001	-0.9863	<0.0001
Linolenate	-0.9314	<0.0001	-0.7892	0.0005
Palmitate				
Stearate	0.1223	0.6643	0.6486	0.0089
Oleate	-0.4483	0.0938	-0.7757	0.0007
Linoleate	0.4229	0.1163	0.7805	0.0006
Linolenate	0.4298	0.1098	0.5165	0.0487
Stearate				
Oleate	-0.2348	0.3996	-0.6432	0.0097
Linoleate	0.2376	0.3938	0.5622	0.0292
Linolenate	0.1202	0.6697	0.6599	0.0074
Oleate (O)				
Linoleate	-0.9986	<0.0001	-0.9841	<0.0001
Linolenate	-0.9502	<0.0001	-0.8463	<0.0001
Linoleate (L)				
Linolenate	0.9352	<0.0001	0.7429	0.0015

^z Corr. = Pearson correlation coefficients, $n = 180$ cotyledons for each soybean line; P = probability that $c > 0$; and O/L = soybean seed oleic:linoleic acid ratio.

decreased linoleic ($P < 0.0001$) and linolenic acid ($P < 0.0001$) concentrations in the seed. The palmitic and stearic acid content in seed of both lines decreased with increasing temperature, but these changes, similar and relatively small in magnitude, were generally significant ($P < 0.006$) only between the 26/22 and 34/26°C treatments. Seed of both lines produced in the cool environment (22/18°C) had similar profiles for all five fatty acids and the O/L ratio. Seed of both lines produced at 26/22 and 34/26°C had similar patterns of change for oleic, linoleic, and linolenic acid contents and the O/L ratio, but the response of N3034-303-3 to increasing temperature was more dramatic compared with the response of N3034-303-7. This difference in response to temperature is illustrated by the O/L ratios achieved with N3034-303-3 (0.42, 0.79, and 1.86) compared with those of N3034-303-7 (0.43, 0.62, and 0.72).

Relationships between ergosterol content of seed colonized with *C. kikuchii* and seed fatty acid content. Colonization by *C. kikuchii* of N3034-303-3 and N3034-303-7 seed with different O/L ratios was quantified by measuring the ergosterol content of inoculated seed as described by Xue et al. (26). For both soybean lines, seed colonization by this pathogen increased with increasing O/L ratio (increasing oleic acid, decreasing linoleic acid; Table 2). Linear correlation analysis (SAS PROC CORR) was conducted for each soybean line to determine how seed fatty acid composition and colonization of seed by *C. kikuchii* were related (Table 3). Ergosterol accumulation for N3034-303-3 was not correlated with palmitic ($P < 0.69$) and stearic ($P < 0.65$) acid but it was positively correlated with the O/L ratio ($r = 0.54$, $P < 0.04$) and the oleic acid content ($r = 0.62$, $P < 0.01$), and it was negatively correlated with linoleic ($r = -0.62$, $P < 0.01$) and linolenic acid content ($r = -0.60$, $P < 0.02$). Results for N3034-303-7 were similar for five of the six fatty acid parameters (Table 3) and showed that ergosterol accumulation in *C. kikuchii*-inoculated seed also was not correlated with palmitic acid, was positively correlated with the O/L ratio and the oleic acid content, and was negatively correlated with the linoleic and linolenic acid contents. N3034-303-7 differed from N3034-303-3 in that ergosterol accumulation also was negatively correlated with stearic acid content ($r = 0.60$, $P < 0.02$).

Relationships between ergosterol content in seed colonized with *D. phaseolorum* and soybean seed fatty acid content. Quantification of *D. phaseolorum* colonization of N3034-303-3 and N3034-303-7 seed with different O/L ratios also was estimated by measuring the ergosterol content of inoculated seed (Table 2). Much larger variations in ergosterol content were observed in seed inoculated with *D.*

phaseolorum compared with seed inoculated with *C. kikuchii* and no clear trends were observed for fungal colonization of seed fatty acid constituents. A linear correlation analysis (SAS PROC CORR) of each soybean line (Table 4) indicated that seed colonization by *D. phaseolorum* was not correlated with any seed fatty acid component or the O/L ratio.

DISCUSSION

We used this approach in an attempt to avoid problems associated with the use of seed from soybean genotypes differing both in genetic background as well as fatty acid profile. Our use of temperature manipulation was based on the results of research demonstrating that temperature can control the fatty acid composition of soybean seed. Field research (9,10,14,15) has shown that oleic, linoleic, and linolenic acid levels of soybean seed are particularly vulnerable to differences in environmental temperature. Controlled growth-chamber experiments (2,22) have shown that linoleic and linolenic acid levels decrease but oleic acid increases as temperature increases. Several mechanisms are likely to be involved in the temperature regulation of fatty acid composition of storage lipid in soybean seed. Higher growth temperatures have been shown to decrease oleate and linoleate desaturase enzyme activity (5) and desaturase gene expression (2), resulting in increased oleic and decreased linoleic or linolenic acid content in seed. In addition, two post-translational

regulatory mechanisms, elevated-temperature-induced instability of the oleate desaturase isoform 1-A and phosphorylation of both oleate desaturase isoforms 1-A and 1-B (19), likely play important roles in modulating the activity of oleate desaturase.

By quantifying seed colonization by measuring the accumulation of the fungal biomass marker ergosterol, we demonstrated that increasing oleic acid content and O/L ratios in seed is related to seed colonization by one of the pathogens, *C. kikuchii*. However, although seed of both soybean lines with higher oleic and lower linoleic (and linolenic) acid content supported a significantly higher level of fungal growth, variation between the two related and highly inbred lines was observed. Seed colonization for almost identical O/L ratios differed between the two lines. At a mean O/L of 0.42, N0304-303-3 ergosterol content was a mean of 55.8 µg/g. At a mean O/L of 0.43, N0304-303-7 ergosterol content was 30.2 µg/g. The O/L ratios achieved by temperature manipulation of the two lines, although of similar trend, differed with a less dramatic numerical range for N0304-303-7 compared with N0304-303-3. In contrast, using the same experimental design and data analysis, we did not establish a clear association between the extent of seed colonization by *D. phaseolorum* and the seed oleic and linoleic acid content. This result initially was surprising because previous research indicated that higher linoleic (and lower

oleic) acid was related to increased seed colonization by this pathogen (23,26). Explanations for the difference in results reported here and in the two previous studies may be that the *D. phaseolorum*-seed interaction is sensitive to genetic background components that differ among the soybean cultivars or the variability in the pathogen isolates used in the different studies.

The comprehensively studied oilseed-fungal pathogen interaction between peanut (*Arachis hypogaea* L.) and *Aspergillus* spp. showed that the effects of fatty acids and fatty acid derivatives on fungal development can be complex. Higher oleic acid content in peanut seed destined for oil extraction is, as for soybean, a desirable trait for producers and consumers. However, laboratory experiments show that peanut seed with high oleic acid content (80% oleate) supported nearly twice as much postharvest production of the polyketide carcinogen, aflatoxin, as normal lines (24,25). Linoleic acid and plant-derived linoleic acid derivatives also have specific effects on the development of *Aspergillus* spp. that are likely to be important for the ability of this pathogen to colonize peanut seed. These effects include the induction of increased sporulation in *Aspergillus flavus* and *A. parasiticus* (Speare) and altered sclerotium production in *A. flavus* (3,4). An *Aspergillus* mutant unable to produce linolenic acid demonstrated delayed spore germination, reduced growth, a reduced level of sporulation, and a complete loss of sclerotial development compared with the wild type (20). Colonization of peanut seed by the mutant was impaired compared with the wild type. Thus, relatively low levels of linoleic acid in the seed could repress seed colonization but stimulate aflatoxin production. In the *C. kikuchii*-soybean seed interaction, higher seed oleate appeared to stimulate seed colonization; however, the effects of oleate and other fatty acids on *Cercospora* sporulation are unknown and will be the subject of future research.

ACKNOWLEDGMENTS

We thank W. Novitzky (United States Department of Agriculture-Agricultural Research Service, North Carolina State University, Raleigh) for the analysis of soybean seed fatty acid composition and the staff of the Southeastern Plant Environmental Laboratory at North Carolina State University for growth chamber space.

LITERATURE CITED

1. Burton, J. W., Carter, T. E., Jr., and Huie, E. B. 1994. Registration of 'Brim' soybean. Crop Sci. 34:301.
2. Byfield, G. E., and Upchurch, R. G. 2007. Effect of temperature on Δ-9 stearoyl-ACP and ω-6 desaturase gene expression and fatty acid content in developing soybean seeds. Crop Sci. 47:1698-1704.
3. Calvo, A. M., Gardner, H. W., and Keller, N. P. 2001. Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*. J. Biol. Chem. 276:25766-25774.
4. Calvo, A. M., Hinze, L. L., Gardner, H. W.,

Table 4. Relationships between the seed fatty acid composition of N0304-303-3 and N0304-303-7 and ergosterol content following inoculation with *Diaporthe phaseolorum*²

Variable 1, variable 2	N0304-303-3		N0304-303-7	
	Corr.	P value	Corr.	P value
Ergosterol				
O/L	-0.1480	0.5987	-0.4323	0.1075
Palmitate	-0.0456	0.8718	0.0759	0.7881
Stearate	-0.2330	0.4032	0.2625	0.3447
Oleate	-0.0311	0.9124	-0.3951	0.1450
Linoleate	0.0587	0.8355	0.4949	0.0607
Linolenate	-0.0882	0.7546	0.0424	0.8807
O/L				
Palmitate	-0.3946	0.1456	-0.5768	0.0244
Stearate	-0.3385	0.2172	-0.6675	0.0066
Oleate	0.9758	<0.0001	0.9953	<0.0001
Linoleate	-0.9809	<0.0001	-0.9779	<0.0001
Linolenate	-0.9073	<0.0001	-0.7255	0.0022
Palmitate				
Stearate	0.2746	0.3219	0.8218	0.0002
Oleate	-0.3726	0.1714	-0.6221	0.0133
Linoleate	0.3349	0.2224	0.4611	0.0837
Linolenate	0.4131	0.1260	0.6577	0.0077
Stearate				
Oleate	-0.4093	0.1298	-0.7074	0.0032
Linoleate	0.4011	0.1384	0.5630	0.0289
Linolenate	0.3692	0.1757	0.6858	0.0048
Oleate (O)				
Linoleate	-0.998	<0.0001	-0.9561	<0.0001
Linolenate	-0.968	<0.0001	-0.7823	0.0006
Linoleate (L)				
Linolenate	0.9519	<0.0001	0.5734	0.0255

² Corr. = Pearson correlation coefficients, *n* = 180 cotyledons for each soybean line; *P* = probability that *c* > 0; and O/L = soybean seed oleic:linoleic acid ratio.

- and Keller, N. P. 1999. Sporogenic effect of polyunsaturated fatty acids on development of *Aspergillus* spp. *Appl. Environ. Microbiol.* 65:3668-3673.
5. Cheesbrough, T. M. 1989. Changes in enzymes for fatty acid synthesis and desaturation during acclimation of developing soybean seeds to altered growth temperature. *Plant Physiol.* 90:760-764.
6. Doehlert, D. C., Wicklow, D. T., and Gardner, H. W. 1993. Evidence implicating the lipoxygenase pathway in providing resistance to soybeans against *Aspergillus flavus*. *Phytopathology* 83:1473-1477.
7. Ehrenshaft, M., and Upchurch, R. G. 1993. Host protein(s) induces accumulation of the toxin cercosporin and mRNA in a phytopathogenic strain of *Cercospora kikuchii*. *Physiol. Mol. Plant Pathol.* 43:95-107.
8. Gilbert, J., Abramson, D., McCallum, B., and Clear, R. 2001. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. *Mycopathologia* 153:209-215.
9. Hou, G., Ablett, G. R., Pauls, K. P., and Rajcan, I. 2006. Environmental effects on fatty acid levels in soybean oil. *J. Am. Oil Chem. Soc.* 83:759-763.
10. Neidلمان, S. L. 1987. Effects of temperature on lipid unsaturation. *Biototechnol. Genet. Eng. Rev.* 5:245-268.
11. Orth, C. E., and Schuh, W. 1994. Resistance of 17 soybean cultivars to foliar, latent, and seed infection by *Cercospora kikuchii*. *Plant Dis.* 78:661-664.
12. Pathan, M. A., Sinclair, J. B., and McClary, R. D. 1989. Effects of *Cercospora kikuchii* on soybean seed germination and quality. *Plant Dis.* 73:720-723.
13. Phytotron Procedural Manual, Technical Bulletin 244 (revised), January 2008, p.16, available on line at www.ncsu.edu/phytotron/manual.pdf.
14. Rajcan, I., Hou, G., and Weir, A. D. 2005. Advances in breeding of seed-quality traits in soybean. *J. Crop Improv.* 14:145-174.
15. Rennie, B. D., and Tanner, J. W. 1989. Fatty acid composition of oil from soybeans seeds grown at extreme temperatures. *J. Am. Oil Chem. Soc.* 66:1622-1624.
16. Sinclair, J. B. 1993. Phomopsis seed decay of soybeans—a prototype for studying seed disease. *Plant Dis.* 77:320-334.
17. Sinclair, J. B. 1995. Reevaluation of grading standards and discounts for fungus-damaged soybean seeds. *J. Am. Oil Chem. Soc.* 72:1415-1419.
18. Sinclair, J. B. 1999. Diaporthe–Phomopsis complex. Page 31 in: *Compendium of Soybean Diseases*, 4th ed. G. L. Hartman, J. B. Sinclair, and P. A. Blackman, eds. APS Press, The American Phytopathological Society, St. Paul, MN.
19. Tang, G.-Q., Novitzky, W. P., Griffin, H. C., Huber, S. C., and Dewey, R. E. 2005. Oleate desaturase enzymes of soybean: evidence of regulation through differential stability and phosphorylation. *Plant J.* 44:433-446.
20. Wilson, R. A., Calvo, A. M., Chang, P.-K., and Keller, N. P. 2004. Characterization of the *Aspergillus parasiticus* Δ^{12} -desaturase gene: a role for lipid metabolism in the *Aspergillus*–seed interaction. *Microbiology* 150:2881-2888.
21. Wilson, R. F. 2004. Seed Composition. Pages 621-677 in: *Soybeans: Improvement, Production, and Uses*. H. R. Boerma and J. E. Specht, eds. American Society of Agronomy Publishers, Madison, WI.
22. Wolf, R. B., Cavins, J. F., Kleiman, R., and Black, L. T. 1982. Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids, and sugars. *J. Am. Oil Chem. Soc.* 59:230-232.
23. 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.
24. Xue, H. Q., Isleib, T. G., Payne, G. A., Novitzky, W. P., and O'Brian, G. 2005. Aflatoxin production in peanut lines selected to represent a range of linoleic acid concentrations. *J. Food Prot.* 68:126-132.
25. Xue, H. Q., Isleib, T. G., Payne, G. A., Wilson, R. F., Novitzky, W. P., and O'Brian, G. 2003. Comparison of aflatoxin production in normal- and high-oleic backcross-derived peanut lines. *Plant Dis.* 87:1360-1365.
26. Xue, H. Q., Upchurch, R. G., and Kwanyuen, P. 2006. Ergosterol as a quantifiable biomass marker for *Diaporthe phaseolorum* and *Cercospora kikuchii*. *Plant Dis.* 90:1395-1398.
27. Zeile, W. L., Knauff, D. A., and Kelly, C. B. 1993. A rapid non-destructive technique for fatty acid determination in individual peanut seed. *Peanut Sci.* 20:9-11.