JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Interrelationship of Phytoalexin Production and Disease Resistance in Selected Peanut Genotypes

VICTOR S. SOBOLEV,*,† BAOZHU Z. GUO,‡ C. CORLEY HOLBROOK,§ AND ROBERT E. LYNCH‡

National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 509, Dawson, Georgia 39842, Crop Protection and Management Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 748, Tifton, Georgia 31793, and Crop Genetics and Breeding Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 748, Tifton, Georgia 31793

In peanuts, a mechanism of resistance to fungal infection is reportedly due to the synthesis of stilbene phytoalexins, which are antibiotic, low molecular weight metabolites. The phytoalexin-associated response of different peanut genotypes to exogenous invasion in the field has not been investigated and may be useful for breeding resistant peanut cultivars. Five peanut genotypes, Georgia Green, Tifton 8, C-99R, GK-7 High Oleic, and MARC I, which differ in resistance to major peanut diseases, were investigated for their ability to produce phytoalexins under field conditions in South Georgia in 2001 and 2002. Five known peanut phytoalexins, *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, were quantitated. The phytoalexins were measured in peanuts of different pod maturity (yellow, orange, brown, and black) with or without insect pod damage (externally scarified or penetrated). Kernels from insect-damaged pods of C-99R and Tifton 8 genotypes had significantly higher concentrations of phytoalexins than other genotypes. The same genotypes were the most resistant to tomato spotted wilt virus and late leaf spot, while MARC I, which is highly susceptible to these diseases, produced very low concentrations of phytoalexins. However, there was no significant difference in phytoalexin production by undamaged peanut pods of all tested genotypes. *trans*-Arachidin-3 and *trans*-resveratrol were the major phytoalexins produced by insect-damaged peanuts. In damaged seeds, the concentrations of *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene were significantly higher in Tifton 8 as compared to other genotypes. There was an association between total phytoalexin production and published genotype resistance to major peanut diseases. Stilbene phytoalexins may be considered potential chemical markers in breeding programs for disease-resistant peanuts.

KEYWORDS: Peanut; *Arachis hypogaea*; groundnut; phytoalexin; stilbene; resveratrol; arachidin; lesser cornstalk borer; drought stress; disease resistance; pod maturity; HPLC-MS

INTRODUCTION

Pathogens attack all parts of the peanut plant throughout the growing season, restricting normal peanut growth and development (*1*). Most of the pathogens that attack peanuts are of fungal origin (*2*). Resistance of peanut kernels to fungal infection has been associated with the kernel's capacity to synthesize antibiotic stilbene phytoalexins in response to injury and infection by certain pathogens, including *Aspergillus flavus* and *A. parasiticus* (*3*−*7*). Dorner et al. (*6*) demonstrated that, as the water content of peanut seed decreased during drought and temperature stresses, the capacity of seeds to produce phytoalexins declined, resulting in fungal infection and aflatoxin contamination. Late-season drought can lead to dangerous aflatoxin contamination (*6*, *8*, *9*) and insect injury, especially by the lesser cornstalk borer (LCB), which can exacerbate aflatoxin contamination in peanuts (*8*, *10*). Invasion by *A. flavus* and *A. parasiticus* and aflatoxin production in peanuts subjected to water-stress first occurs in small, immature peanuts (*6*), suggesting that mature peanut pods possess resistance mechanisms inhibiting fungal growth (*11*). However, the exact mechanism of peanut resistance to fungal invasion is not understood. A greater understanding of peanut resistance to pests can be significant if it uncovers fundamental biological mechanisms of resistance. For many diseases, highly effective resistance is not known, so quantitatively expressed resistance is being sought.

* Author to whom correspondence should be addressed [telephone (229) 995-7446; fax (229) 995-7416; e-mail vsobolev@nprl.usda.gov].
† National Peanut Laboratory.
‡ Crop Protection Unit.
§ Crop Genetics Unit.

10.1021/jf063478g
Published on Web 02/28/2007

Of over 100 possible high-impact insects on peanuts (*2*), the lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* Zeller, is one of the most destructive throughout the area of the United States where peanuts are produced (*12*−*14*). It is considered a dry land insect because damage occurs most often in light, sandy soil and tends to be more severe during periods of drought (*14*). Penetration of peanut pods by insects enhances infection of pods by *A. flavus* and *A. parasiticus* and aflatoxin contamination in peanut seeds before and after harvest, and during transportation and storage (*8*, *9*). However, no more than external scarification of the pod is necessary for increased *A. flavus* infection (*10*). Aflatoxin concentrations in seeds from pods injured by insects can be dramatically higher than those in the seeds from uninjured pods. Insect injury to peanut pods may result in aflatoxin contamination in seeds under conditions that normally do not favor fungal infection and aflatoxin production in uninjured pods. Conditions that favor LCB damage to peanut pods, that is, drought and high soil temperatures, are similar to conditions that favor infection of pods by *A. flavus* and *A. parasiticus* (*10*, *15*).

Despite the large number of cultivars available to growers, the peanut crop has been characterized as being genetically vulnerable to diseases and insect pests (*16*, *17*). Throughout the 20th century, scientists have exploited natural resistance to improve crop varieties. As a result, breeding for plant resistance to plant pathogens and insects has been one of the major achievements in alleviating the impact of several important diseases in peanuts. Host plant resistance is an efficient, economical, and environment-friendly approach used to manage many pests and diseases of agricultural crops. Efficient selection for resistance during the breeding process has been facilitated by linkages to markers (*18*). Chemical markers can be useful to combine genes with similar resistance phenotypes. Understanding the processes triggering resistance will be useful for manipulating resistance genes for greater effectiveness and stability of resistance.

Runner-type cultivars have become the dominant peanut type grown after the introduction in the 1970s of a new cultivar, Florunner, which was responsible for a dramatic increase in peanut yields (*2*). The current dominant cultivar is Georgia Green, which became popular because of its field resistance to tomato spotted wilt virus (TSWV) (*2*). During the 2002 growing season, over 90% of the peanut acreage in Georgia was planted to Georgia Green (*19*), and, at present, runner-type cultivars account for 80% of total production in the U.S. (*2*). Susceptibility of peanuts to TSWV is one of the major issues in the U.S. peanut industry.

The objective of this research was to study the phytoalexin production by resistant and susceptible peanut genotypes in the field as influenced by lesser cornstalk borer damage and drought stress.

MATERIALS AND METHODS

Peanut Genotypes. Four runner types, Georgia Green, C-99R, MARC I, and GK7 High Oleic, and a Virginia type, Tifton 8, were made available by the Crop Genetics and Breeding Research Unit, USDA, Tifton, GA.

Procedure. Peanuts were grown on a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) in Tift County, Southwest Georgia. Peanuts were planted in early May in 2001 and 2002. Irrigation was applied as needed for the first 90 days. The insecticide Lorsban was applied to the plots 45 days after plant emergence according to conventional agricultural practices in Georgia.

Drought Stress. Drought stress was induced by covering three 30′ × 90′ test fields with a mobile rainout shelter (Atlas Greenhouse System Inc., Alapaha, GA). Distance between fields was 5′. The rainout shelters were moved over the experimental fields at the 90th day after peanut planting. One control 30′ × 90′ field was set between the experimental fields. Peanut samples from the control field were collected during the drought-stress experiments to monitor kernel water activity (a_w).

Lesser Cornstalk Borer Infestation. Beginning 1 week after inducing drought, all plots were artificially infested with lesser cornstalk borer larvae in vermiculite. Infestation was continued three times a week at equal intervals for 3 weeks.

Sampling of Peanuts. Six replicates of each peanut genotype were collected by hand-digging of the whole peanut plots (5 ft long twin row plot) on the 118th day after planting in 2001 and on the 125th day in 2002. The pods were placed separately in labeled plastic bags and brought to the laboratory without delay. Samples were separated into undamaged and damaged (scarified and penetrated combined) classes on the basis of lesser cornstalk borer damage to the peanut pods, and maturity of the pods was determined as described below.

Determination of Water Activity (a_w). For a_w measurements, 10 pods per maturity stage per genotype were hand-shelled, kernels split, and placed in sample dishes until measurements were accomplished at 25 ± 0.5 °C with a series 3TE AquaLab water activity meter (Decagon Devices, Inc., Pullman, WA). The instrument was calibrated using salt slurries according to the instrument instructions. The measurements were performed every seventh day beginning 1 week prior to moving the rainout shelters over the experimental and control fields.

Determination of Maturity Stages. Pods were placed in a wet impact blaster (*20*) to remove the exocarp and expose the color of mesocarp. Color in the mesocarp was the basis for the hull-scrape maturity classification method (*21*). Pods were then divided into different colors (yellow, orange, brown, and black) as to maturity and stored in a freezer at −20 °C for later analysis. Insect-damaged pods were not blasted due to the holes in the pods and therefore were not divided into maturity groups; if blasted with glass beads under high water pressure, the pods became contaminated from inside with soil and the exocarp debris.

Reference Compounds. *trans*-Resveratrol (approximately 99%) was purchased from Sigma. Pure individual peanut stilbenes, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, were obtained as described previously (*22*). In brief, peanut kernels were allowed to imbibe water for 18 h. The kernels were then ground in a food processor to obtain 3−5 mm size pieces followed by their inoculation with an *A. flavus* strain and incubation without light at 23−26 °C and close to 100% relative humidity for 5 days. After incubation, the peanuts were extracted with a CH_3CN-H_2O mixture in a high-speed blender (General Electric, New York, NY). The extract was filtered, and the filtrate was placed in a freezer for 18 h to remove water by freezing. The extract was vacuum-filtered, and the solvent was removed from the filtrate on a rotary evaporator (Rotavapor-R, Brinkmann Instruments, Westbury, NY) at 40 °C. The residue was subjected to chromatography on a silica gel column. The column was eluted with a gradient of *n*-hexane−CH_3COCH_3. Eluates from the column were analyzed by TLC. Fractions containing phytoalexins with identical R_f values were combined and evaporated on a rotary evaporator. Dry residues were purified using preparative HPLC. The separation was achieved by using a 100 × 19 mm i.d., 5 μm XTerra Prep RP_{18} OBD preparative HPLC column (Waters). H_2O (A), MeOH (B), and 1% HCOOH in H_2O (C) were combined in the following gradient: initial conditions, 65% A/30% B/5% C, held for 1 min, increased linearly to 0% A/95% B/5% C in 12 min, held isocratic for 5 min, decreased to initial conditions in 0.01 min. The flow rate was 9.9 mL/min. Combined eluates containing pure corresponding compound were diluted with distilled water and extracted three times with EtOAc. Combined EtOAc layers were evaporated to dryness with a rotary evaporator. The compounds were obtained as white or yellowish−off-white microcrystalline solids. The crystals were dried for 48 h in a desiccator with dry silica gel. The identity of the reference compounds as well as stilbenes extracted from peanuts was confirmed by ESI-MS/MS (MS^2) and UV spectroscopy. The values determined in this research are given in parentheses as $[M + H]^+$ values followed by UV absorption maxima: *trans*-resveratrol (*m*/*z* 229; 305 and 317 nm), *trans*-arachidin-1 (*m*/*z* 313; 339 nm), *trans*-arachidin-2

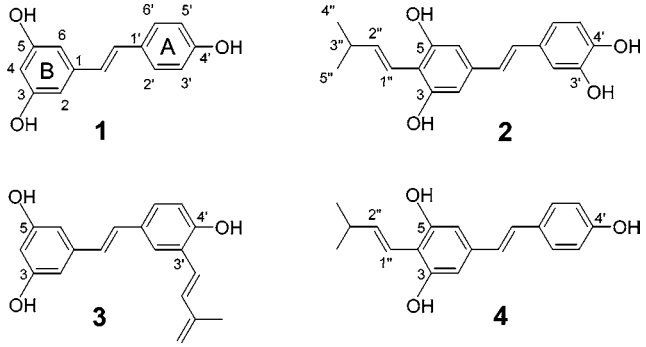

Figure 1. Structures of stilbene phytoalexins found in tested peanut genotypes. 1, *trans*-resveratrol; 2, *trans*-arachidin-1; 3, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene; 4, *trans*-arachidin-3.

(*m*/*z* 297; 308 and 322 nm), *trans*-arachidin-3 (*m*/*z* 297; 334 nm), and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene (*m*/*z* 295; 296 nm). The above results were in agreement with published data (*4*, *5*, *7*, *22*, *24*−*26*).

Extraction of Phytoalexins from Peanut Kernels. Samples from the different maturity classes were shelled by hand. Ten grams of peanut kernels was placed in a blender jar with 30 mL of CH_3CN-H_2O (90 + 10, v/v) and blended for 1 min at high speed. The extract was filtered through a glass fiber filter. One milliliter of the filtrate was evaporated to dryness with a nitrogen stream in a model 18780 Reacti-Vap evaporating unit at 40 °C (Pierce, Rockford, IL). The residue was dissolved in 0.3−5.0 mL of the HPLC mobile phase; the volume of the solvent was chosen on the basis of the expected level of phytoalexins (*27*). Next, 5−50 μL of the extract was injected into HPLC. Concentrations of *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-3, and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene were determined by reference to peak areas of corresponding pure standards at 317, 339, 334, and 296 nm, respectively.

HPLC-Diode Array Detection-MS Analyses of Stilbene Phytoalexins. Analyses were performed using an HPLC system equipped with a LC-10ATvp pump (Shimadzu, Kyoto, Japan), a SPD-M10Avp diode array detector covering the 200−500 nm range with Shimadzu Client/Server software, version 7.3, and a model 717 plus autosampler (Waters, Milford, MA). The separation was performed on a 50 mm × 4.6 mm i.d., 2.5 μm XTerra MS C18 analytical column (Waters). H_2O (A), MeOH (B), and 1% HCOOH in H_2O (C) were combined in the following gradient: initial conditions, 95% A/0% B/5% C, increased linearly to 0% A/95% B/5% C in 15 min, held isocratic for 1 min, decreased to initial conditions in 0.01 min. The flow rate was 1.2 mL/min. The column was maintained at 35 °C in a model 105 column heater (Timberline Instruments, Boulder, CO). The eluate from the diode array detector was split with a T-unit (Upchurch Scientific, Oak Harbor, WA) for optimal MS performance. Flow rate through the ESI probe was set at 0.35 mL/min. MS analyses were performed using a Finnigan LCQ Advantage MAX ion trap mass spectrometer equipped with an ESI interface and operated with Xcalibur version 1.4 software (Thermo Electron Corp., San Jose, CA). The data were acquired in the full-scan mode (MS) from *m*/*z* 100 to 2000. Heated capillary temperature was 200 °C, sheath gas flow 30 units, capillary voltage 13 V, and source voltage 4.5 kV. In MS^2 analyses, the $[M + H]^+$ ions observed for each chromatographic peak in full-scan analyses were isolated and subjected to source collision-induced dissociation (CID) using He buffer gas. In all CID analyses, the isolation width, relative fragmentation energy, relative activation *Q*, and activation time were *m*/*z* = 1.5, 25% or 30%, 0.25, and 30 ms, respectively.

Data Analysis. Data were analyzed by ANOVA procedures using SAS, 2000 (SAS Institute, Inc., Version 7, Cary, NC). Multiple comparisons of the various means were carried out by least significant difference (LSD) test at $p = 0.05$. Pearson product−moment correlation coefficient was calculated using SigmaStat (version 3.1, SYSTAT Software, Inc., Point Richmond, CA). Comparison of means of two groups of data was performed using the *t*-test; the Mann−Whitney Rank Sum Test was applied when the normality test failed ($p < 0.050$).

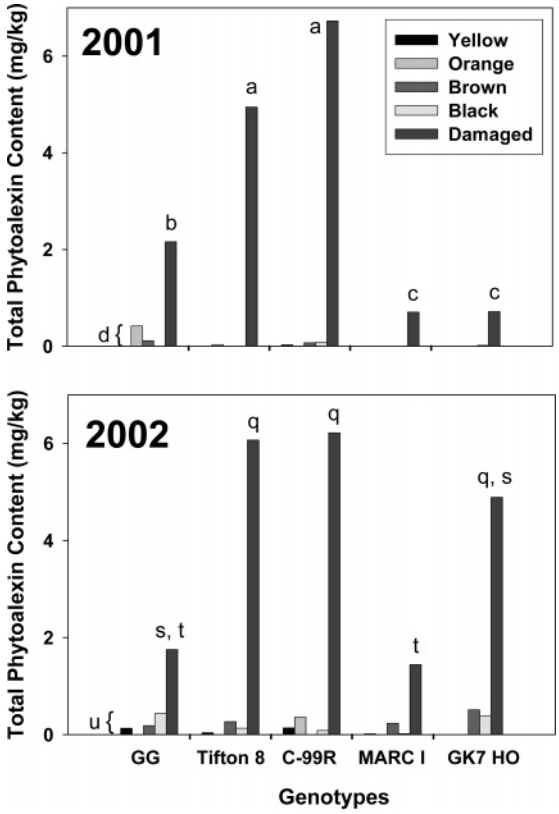

Figure 2. Total phytoalexin production by kernels of undamaged pods of tested peanut genotypes of different maturities and insect-damaged pods regardless of their maturities. GG, Georgia Green; GK-7 HO, GK-7 High Oleic peanut genotypes. Means followed by the same letter are not significantly different, $p = 0.05$. All means in yellow, orange, brown, and black maturity groups are not significantly different (d, u). Six replicates of damaged peanuts and six replicates of peanuts from each maturity group of each genotype (total of 150 samples per year) are represented.

RESULTS AND DISCUSSION

It has been demonstrated (*6*) that, as long as peanuts had the capacity for phytoalexin production, they were not contaminated with aflatoxins. We suggested that not only resistance to preharvest aflatoxin contamination (PAC) (*6*, *27*), but also resistance to some common peanut diseases might be associated with phytoalexin production. When different peanut genotypes, grown in the same experimental field, undergo a controlled drought stress, they may show different signs of weakness or strength: they may die, become infested by pests, or remain intact depending on their genetic ability to resists exogenous invasions. Therefore, any measurable response by peanut genotypes, including phytoalexin production, to such stress may help to identify and estimate peanut resistance to diseases. Based on published data (*6*), the capacity to produce phytoalexins in peanuts was lost in all maturity stages between 24 and 32 heat treatment days. In our drought experiments, we intended to initiate heat and drought stress without reducing peanut ability to synthesize phytoalexins (**Figure 1**). We used the above information (*6*) as well as the data obtained from our preliminary experiments (*27*) for the drought-stress design for this study. Desired degree of drought stress was assured by monitoring water activity (a_w) of kernels of tested genotypes collected from the control field. Water activity levels were not significantly different between the genotypes ($n = 160$, $p < 0.05$). During the first 3 weeks of drought, a_w of kernels stayed in the 0.95−0.98 range both in 2001 and in 2002. However, average kernel

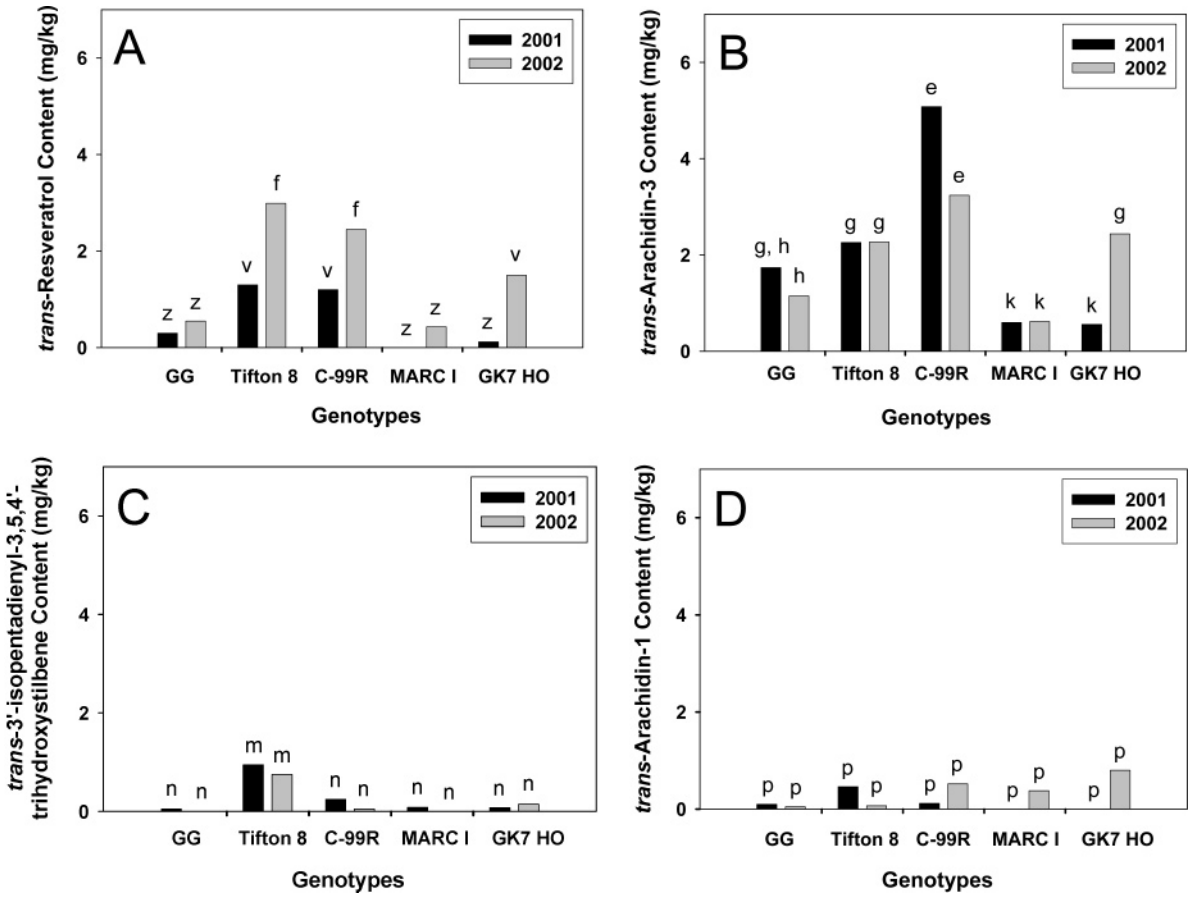

Figure 3. Production of phytoalexins by insect-damaged kernels regardless of their maturities: **A**, *trans*-resveratrol; **B**, *trans*-arachidin-3; **C**, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene; **D**, *trans*-arachidin-1. GG, Georgia Green; GK-7 HO, GK-7 High Oleic peanut genotypes. Means followed by the same letter are not significantly different, $p = 0.05$.

a_w at the end of drought experiments dropped to 0.931 ± 0.012 and 0.936 ± 0.008 in 2001 and 2002, respectively. These data allow one to suggest that experimental peanuts grown under similar drought conditions had similar a_w values. The patterns of a_w in 2001 and 2002 were similar to previously reported patterns (*6*, *27*), when a positive relationship of phytoalexin production to kernel a_w was observed.

Runner genotypes were chosen as the most accepted in major peanut-growing areas in the U.S. One Virginia germplasm line, Tifton 8, was used for comparison as one of the most drought- and PAC-resistant genotypes (*28*, *29*). A possible PAC resistance mechanism of Tifton 8 was attributed to the capacity to maintain high water activity in the kernel due to a larger root system (*27*) as compared to other genotypes.

In 2002, overall phytoalexin production by all tested peanut genotypes in all maturity groups (excluding damaged) was significantly higher ($n = 120$, $p < 0.001$) than that in 2001 (**Figure 2**). Such differences in data are commonly observed on a yearly basis in replicate field experiments. However, the production patterns for *trans*-resveratrol and *trans*-arachidin-3 (**Figure 3A** and **B**) by damaged kernels of all genotypes were similar in both 2001 and 2002. Strong correlation of the data on total average phytoalexin production ($r = 0.95$, $p < 0.05$ for all genotypes excluding GK-7 High Oleic) obtained for the two consecutive years (**Figure 2**) allowed us to use the data for some basic conclusions. Analyses indicated that lesser cornstalk borer (LCB) damage significantly increased phytoalexin synthesis by both resistant and susceptible genotypes (**Figure 2**). Kernels from insect-damaged pods of C-99R and Tifton 8 genotypes had higher concentrations of phytoalexins than other genotypes (**Figure 2**). These same genotypes (**Table 1**) were the most resistant to tomato spotted wilt virus (TSWV) and late leaf spot (LLS), while MARC I, which is highly susceptible to these diseases, produced very low concentrations of phytoalexins (**Figure 2**). Resistance to a fungal disease, White Mold, as well as resistance to damage by corn earworm (**Table 1**) were also directly associated with phytoalexin production. Limited published data do not permit comparison of all peanut genotypes for the major diseases; however, all tested genotypes can be directly compared for TSWV and LLS resistance (**Table 1**). The concentrations of phytoalexins in **Table 1** are shown as "high", "medium", and "low" for ease of comparison with the disease ratings. Data in **Table 1** are expressed subjectively in accordance with published objective measurements and subjective observations. Resistance to diseases may vary significantly in irrigated, non-irrigated, and drought-stressed peanut fields. Duration of growth, geographical location, and other factors may be critical as well. However, resistant and susceptible genotypes can be definitely identified when grown side by side. For instance, overall disease incidence and severity for C-99R and Georgia Green was reported as significantly lower than that for MARC I under normal and drought conditions. On the basis of their capacity to produce phytoalexins under drought stress in 2001 (**Figure 2**), tested genotypes can be grouped in descending order: C-99R, Tifton 8, Georgia Green, GK-7 High Oleic, and MARC I. In 2002, the position of Georgia Green and GK-7 High Oleic was reversed, but C-99R and Tifton 8 genotypes showed consistently high production of total phytoalexins at about 6 mg/kg level. MARC I was still the lowest producing (about 1 mg/kg) genotype (**Figure 2**).

Table 1. Compiled Data on Selected Peanut Genotype Disease Resistance[a]

	peanut diseases					field behavior			
peanut genotype	TSWV	WM	LLS	CBR	CEW	PAC	drought tolerance	visual stress	phytoalexin production (present study)
C-99R	M	M	M	S		medium/low	medium	medium	high
Tifton 8	M/L		M		H	medium	high	low	high
Georgia Green	M/L	M/L	L/S	L/S	S	medium/low	medium	medium	medium
GK-7 High Oleic	L	S	S	L/S		medium/low	medium	medium	medium
MARC I	S	S	S	L/S		medium/low	medium	medium	low

[a] TSWV, tomato spotted wilt virus; WM, White Mold (*Sclerotium rolfsii*); LLS, Late Leafspot (*Cercospora personatum*); CBR, Cylindrocladium Black Root (*Cylindrocladium crotalariae*); CEW, corn earworm (*Helicoverpa zea*); PAC, preharvest aflatoxin contamination; H, high resistance; M, moderate resistance; L, low resistance; S, susceptible. Empty cells mean lack of data or limited data from a single source.

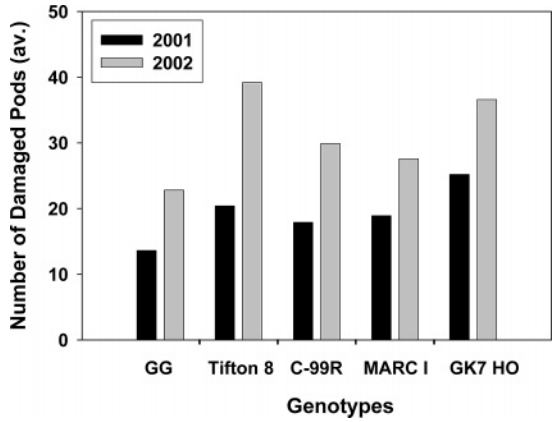

Figure 4. Frequency of peanut pod damage by LCB in tested genotypes. There was a significant difference between total number of damaged pods of all genotypes in 2001 and 2002 (61.5% vs 100%, respectively; $n = 5$, $p = 0.009$). There was no significant difference between peanut genotypes both in 2001 ($n = 5$, $p = 0.181$) and in 2002 ($n = 5$, $p = 0.102$).

Differences among genotypes were only apparent in LCB-damaged peanuts. In undamaged pods, production of phytoalexins was very low. The average number of damaged by LCB peanut pods (six replicates) of tested genotypes in 2001 and 2002 is shown in **Figure 4**. There was a significant difference between combined average numbers of damaged pods of all tested genotypes in 2001 and 2002 (61.5% vs 100%, respectively; $n = 5$, $p = 0.009$). However, there was no significant difference between the genotypes in terms of frequency of insect damage in 2001 ($n = 5$, $p = 0.181$) and 2002 ($n = 5$, $p = 0.102$). This fact allows one to suggest that LCB had equal ability to infest tested genotypes under the experimental conditions. At the same time, the genotypes demonstrated different capacities to produce phytoalexins in response to the pod damage (**Figures 2** and **3**). These data may indicate that the observed differences in phytoalexin production were genotype-related and were not associated with the rate of insect damage.

There was no significant difference among peanut maturity groups (excluding damaged) in terms of phytoalexin production in 2001 and 2002. The major phytoalexin found in damaged pods in 2001 and 2002 was *trans*-arachidin-3 (**Figure 3B**). The second highest phytoalexin was *trans*-resveratrol (**Figure 3A**). *trans*-Resveratrol in 2001 was significantly higher in Tifton 8 and C-99R as compared to other varieties (**Figure 3**). In 2002, *trans*-resveratrol was produced in significantly higher concentrations by Tifton 8, C-99R, and GK-7 High Oleic. Concentrations of *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene were significantly higher in Tifton 8 than other genotypes, reaching on average 0.94 mg/kg (**Figure 3C**). Whether this phytoalexin is important for the genotype resistance is a question to be addressed in future studies. *trans*-Arachidin-1 production (**Figure 3D**) was very low among tested genotypes; the highest concentration was detected in GK-7 High Oleic genotype in 2002 (0.73 mg/kg). *trans*-Arachidin-1 production was random; this phytoalexin does not seem to be a key defensive stilbenoid under the experimental conditions. *trans*-Arachidin-2 was not detected in any of the analyzed samples.

There was an association between total phytoalexin production and published genotype resistance to TSWV and LLS diseases (**Table 1**). In peanut kernels, the mechanism of resistance to fungal infection is reportedly due to the synthesis of stilbene phytoalexins (*3*−*6*) as demonstrated by the high *Cercospora personatum* resistance of high phytoalexin producers, C-99R and Tifton 8 genotypes. Besides kernels, fungus-challenged vegetative parts of the peanut plant such as roots, pegs, stems, and leaves produced the same set of stilbene phytoalexins (*22*), and unchallenged root mucilage accumulated extremely high concentrations of stilbenoids (*30*). The presence of stilbenoids in organs of the peanut plant allows for suggestion of their protective role for the entire plant. There is no evidence that stilbene phytoalexins are directly involved in peanut resistance to viral diseases such as TSWV. However, the ability to synthesize high levels of phytoalexins might be an indication of genetically inherited vigor resulting in superior growth and yield. Such peanuts may have greater ability to maintain multiple physiological resistance functions under challenged conditions. This suggestion agrees with the data obtained by Lyerly et al. (*31*) and Rowland et al. (*32*), who demonstrated that a possible TSWV tolerance mechanism is based on the ability to maintain near-normal photosynthetic levels in symptomless tissue, even in the presence of viral infection (*32*). Such ability may be a more important resistance mechanism than actual avoidance of infection because of the near omnipresence of TSWV in the USA peanut-producing regions (*31*).

In conclusion, the experiments demonstrated that tested peanut genotypes had different abilities to produce phytoalexins under drought stress as influenced by insect damage in the field.

Phytoalexin production was highest in insect-damaged peanut pods. Damaged pods of the most resistant peanut genotypes to TSWV and LLS consistently produced higher concentrations of phytoalexins as compared to susceptible genotypes. There was no significant difference in phytoalexin production by undamaged peanut pods of all tested genotypes. *trans*-Arachidin-3 and *trans*-resveratrol were the major phytoalexins produced by insect-damaged pods of all tested peanut genotypes.

Therefore, these compounds seem to be the stilbenoids of choice when investigating peanut genotypes for their ability to synthesize phytoalexins, and possibly for overall peanut resistance to diseases. Stilbene phytoalexins may be considered potential chemical markers in breeding programs for disease-resistant peanuts.

ACKNOWLEDGMENT

We thank Gareth Sheppard for his help with statistical analyses of the experimental data and Joe Dorner for advice and discussions. We also thank Ernest Harris, Lloyd Copeland, and Robert Giddens for technical assistance in the field and laboratory.

Supporting Information Available: List of references (*33*−*72*) supporting compiled data in **Table 1**. This material is available free of charge via the Internet at http://pubs.asc.org.

LITERATURE CITED

(1) Porter, D. M.; Smith, D. H.; Rodriguez-Kabana, R. Peanut Diseases. In *Peanut Science and Technology*, Pattee, H. E., Young, C. T., Eds.; Am. Peanut Res. Educ. Soc. Inc.: Yoakum, TX, 1982; pp 326−410.

(2) Report on the status of *Arachis* germplasm in the United States. http://www.ars-grin.gov/npgs/cgc_reports/Status11.pdf (accessed: 01/11/2007).

(3) Wotton, H. R.; Strange, R. N. Increased susceptibility and reduced phytoalexin accumulation in drought-stressed peanut kernels challenged with Aspergillus flavus. *Appl. Environ. Microbiol.* **1987**, *53*, 270−273.

(4) Keen, N. T.; Ingham, J. L. New stilbene phytoalexins from American cultivars of Arachis hypogaea. *Phytochemistry* **1976**, *15*, 1794−1795.

(5) Aguamah, G. A.; Langcake, P.; Leworthy, D. P.; Page, J. A.; Pryce, R. J.; Strange, R. N. Two novel stilbene phytoalexins from Arachis hypogaea. *Phytochemistry* **1981**, *20*, 1381−1383.

(6) Dorner, J. W.; Cole, R. J.; Sanders, T. H.; Blankenship, P. D. Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. *Mycopathologia* **1989**, *105*, 117−128.

(7) Wotton, H. R.; Strange, R. N. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to Aspergillus flavus. *J. Gen. Microbiol.* **1985**, *131*, 487−494.

(8) Blankenship, P. D.; Cole, R. J.; Sanders, T. H.; Hill, R. A. Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by Aspergillus flavus and subsequent aflatoxin contamination. *Mycopathologia* **1984**, *85*, 69−74.

(9) Hill, R. A.; Blankenship, P. D.; Cole, R. J.; Sanders, T. H. Effect of soil moisture and temperature on preharvest invasion of peanuts by the Aspergillus flavus group and subsequent aflatoxin development. *Appl. Environ. Microbiol.* **1983**, *45*, 628−633.

(10) Lynch, R. E.; Wilson, D. M. Enhanced infection of peanut, Arachis hypogaea L. seeds with Aspergillus flavus group fungi due to external scarification of peanut pods by the lesser cornstalk borer, Elasmopalpus lignosellus (Zeller). *Peanut Sci.* **1991**, *18*, 110−116.

(11) Cole, R. J.; Sanders, T. H.; Hill, R. A.; Blankenship, P. D. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. *Mycopathologia* **1985**, *91*, 41−46.

(12) Berberet, R. C.; Morrison, R. D.; Wall, R. G. Yield reduction caused by the lesser cornstalk borer in nonirrigated Spanish peanuts. *J. Econ. Entomol.* **1979**, *72*, 525−528.

(13) Smith, J. W., Jr.; Holloway, R. L. Lesser cornstalk borer larval density and damage to peanuts. *J. Econ. Entomol.* **1979**, *72*, 535−537.

(14) Lynch, R. E. Damage and preference of lesser cornstalk borer (Lepidoptera: Pyralidae) larvae for peanut pods in different stages of maturity. *J. Econ. Entomol.* **1982**, *77*, 360−363.

(15) Sanders, T. H.; Cole, R. J.; Blankenship, P. D.; Hill, R. A. Relation of environmental stress duration to Aspergillus flavus invasion and aflatoxin production in preharvest peanuts. *Peanut Sci.* **1985**, *12*, 90−93.

(16) Hammons, R. O. Peanuts: Genetic vulnerability and breeding strategy. *Crop Sci.* **1976**, *16*, 527−530.

(17) Knauft, D. A.; Gorbet, D. W. Genetic diversity among peanut cultivars. *Crop Sci.* **1989**, *29*, 1417−1422.

(18) Holbrook, C. C.; Stalker, H. T. Peanut breeding and genetic resources. *Plant Breed. Rev.* **2003**, *22*, 297−356.

(19) Culbreath, A. K.; Todd, J. W.; Brown, S. L. Epidemiology and management of tomato spotted wilt in peanut. *Annu. Rev. Phytopathol.* **2003**, *41*, 53−75.

(20) Williams, E. J.; Monroe, G. E. Impact blasters for peanut pod maturity determination. *Trans. Am. Soc. Agric. Eng.* **1986**, *29*, 263−266, 275.

(21) Williams, E. J.; Drexler, J. S. A non-destructive method for determining peanut pod maturity. *Peanut Sci.* **1981**, *8*, 131−141.

(22) Sobolev, V. S.; Cole, R. J.; Dorner, J. W.; Yagen, B. Isolation, purification, and liquid chromatographic determination of stilbene phytoalexins in peanuts. *J. AOAC Int.* **1995**, *78*, 1177−1182.

(23) Sobolev, V. S.; Deyrup, S. T.; Gloer, J. B. New peanut (Arachis hypogaea) phytoalexin with prenylated benzenoid and but-2-enolide moieties. *J. Agric. Food Chem.* **2006**, *54*, 2111−2115.

(24) Arora, M. K.; Strange, R. N. Phytoalexin accumulation in groundnuts in response to wounding. *Plant Sci.* **1991**, *78*, 157−163.

(25) Edwards, C.; Strange, R. N. Separation and identification of phytoalexins from leaves of groundnut (Arachis hypogaea) and development of a method for their determination by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **1991**, *547*, 185−193.

(26) Cooksey, C. J.; Garratt, P. J.; Richards, S. E.; Strange, R. N. A dienyl stilbene phytoalexin from Arachis hypogaea. *Phytochemistry* **1988**, *27*, 115−116.

(27) Guo, B. Z.; Sobolev, V.; Holbrook, C. C.; Lynch, R. E. Impact of phytoalexins and lesser cornstalk borer damage on resistance to aflatoxin formation. *Mycopathologia* **2004**, *157*, 476.

(28) Reddy, T. Y.; Reddy, V. R.; Anbumozhi, V. Physiological responses of groundnut (Arachis hypogaea L.) to drought stress and its amelioration: a critical review. *Plant Growth Regul.* **2003**, *41*, 75−88.

(29) Coffelt, T. A.; Hammons, R. O.; Branch, W. D.; Mozingo, R. W.; Phipps, P. M.; Smith, J. C.; Lynch, R. E.; Kvien, C. S.; Ketring, D. L.; Porter, D. M.; Mixon, A. C. Registration of Tifton-8 peanut germplasm. *Crop Sci.* **1985**, *25*, 203.

(30) Sobolev, V. S.; Potter, T. L.; Horn, B. W. Prelylated stilbenes from peanut root mucilage. *Phytochem. Anal.* **2006**, *17*, 312−322.

(31) Lyerly, J. H.; Stalker, H. T.; Moyer, J. W.; Hoffman, K. Evaluation of Arachis species for resistance to Tomato Spotted Wilt Virus. *Peanut Sci.* **2002**, *29*, 79−84.

(32) Rowland, D.; Dorner, J.; Sorensen, R.; Beasley, J. P.; Todd, J. Tomato Spotted Wilt Virus in peanut tissue types and physiological effects related to disease incidence and severity. *Plant Pathol.* **2005**, *54*, 431−440.

Received for review December 1, 2006. Revised manuscript received January 25, 2007. Accepted January 25, 2007.

JF063478G