JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Localized Production of Phytoalexins by Peanut (*Arachis hypogaea*) Kernels in Response to Invasion by *Aspergillus* Species

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Peanuts respond to fungal infection by synthesizing phytoalexins, most of which are antibiotic stilbenes. The mechanism and dynamics of phytoalexin formation in the peanut have not been studied. One of the most popular peanut cultivars in the southeastern United States, Georgia Green, was investigated for its ability to produce phytoalexins in response to infection by soil fungal strains. The experimental design allowed for study of phytoalexin production in peanut kernels layer-by-layer. The layers were dissected from different depths of the kernel starting from the infected area down to healthier tissues. Six peanut phytoalexins, *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene, and SB-1, were detected in the kernel slices and quantitated. All of the fungal strains triggered phytoalexin production; however, the composition of phytoalexins varied significantly by layer. After incubation for 24 h, tissues remote from the infected area produced almost exclusively *trans*-resveratrol, whereas closer to the infected area tissues synthesized all six phytoalexins. In all of the experiments, after 48 h of fungal growth, deeper layers produced all tested phytoalexins. There was a significant difference in phytoalexin production elicited by some fungal isolates. No association was observed between phytoalexin production and toxigenic potential of fungal strains that elicited the production in mature peanut kernels.

KEYWORDS: Peanut; *Arachis hypogaea*; groundnut; phytoalexin; stilbene; resveratrol; arachidin; *Aspergillus* spp.; fungal spores; aflatoxin producer

INTRODUCTION

Peanut can resist infection by pathogens, including *Aspergillus flavus* and *A. parasiticus*, by producing antibiotic stilbene-derived phytoalexins (*1–10*) (**Figure 1**). Sufficient water content in kernels is essential for effective production of the phytoalexins (*9, 10*). However, the mechanism of peanut resistance to exogenous invasion has not been explored.

One of the major peanut stilbenoids, resveratrol is known to exert manifold biological effects that are not limited to the host plant, but found to be beneficial also to human health (*11–14*). Resveratrol is considered in peanut the starting building block for the synthesis of related, less hydrophilic stilbenoids (*7*).

Georgia Green has become the major peanut cultivar grown in the southeastern United States because of its high resistance to tomato spotted wilt virus (*15*). Diverse peanut genotypes grown in the field side-by-side exhibited different responses to fungal attack, producing significantly different amounts of phytoalexins (*16*). It was logical to investigate the reaction of Georgia Green to infection by different fungal species found in peanut field soil.

The objective of this research was to study the phytoalexin production by wounded kernels of a single peanut genotype in response to infection by different fungal strains. An attempt was made to clarify the dynamics of phytoalexin synthesis by analyzing stilbenoid concentrations in dissected kernel slices located at different distances from the infected wound.

EXPERIMENTAL PROCEDURES

Reagents, Materials, and Basic Apparatus. HPLC-grade MeOH used in the preparation of mobile phases and sample extraction was obtained from Fisher (Suwanee, GA). Celite 545 was purchased from J. T. Baker (Phillipsburg, NJ). HPLC-grade H_2O was prepared with a, model ZD20 four-bowl Milli-Q Water System (Millipore). Two percent NaOCl solution was prepared by dilution of household bleach (Clorox). A model 231 Vortex Touch Mixer (Fisher), a series 2000 Adjustable-Volume (100–1000 μL) pipet with matching 1000 μL pipet tips (Eppendorf), a model 815 microtome (American Optical Co., New York) with a high-profile microtome blade (catalog no. 22-244028, Fisher), a type PM 4600 electronic balance (Mettler), 4 mL (15 × 45 mm) clear glass vials (Waters), a 700 μL polypropylene conical autosampler vial with cap (Waters, catalog no. 22476), and a 65 mm diameter agate mortar and matching pestle (Fisher) were used in the research. A Preval sprayer (Precision Valve Corp., Yonkers, NY) was used to disperse fungal spores.

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10.1021/jf703595w
Published on Web 02/26/2008

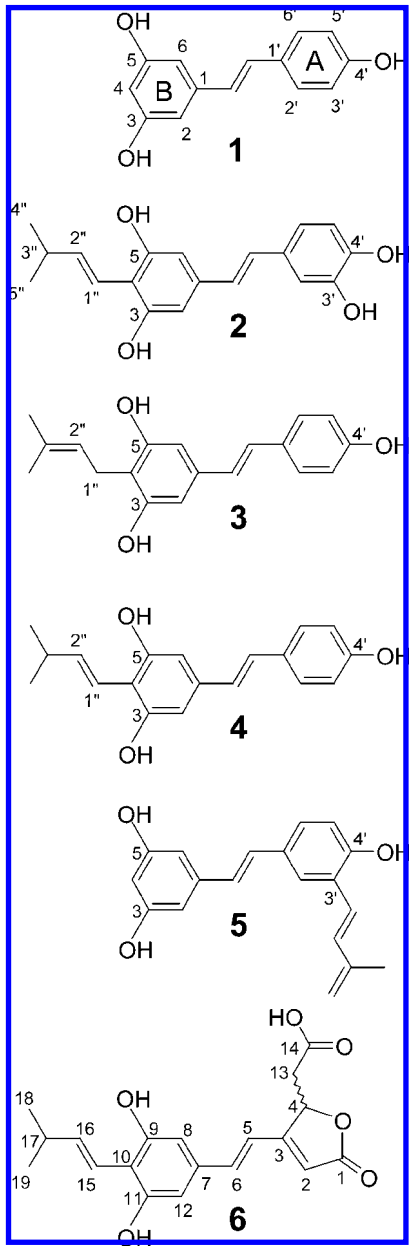

Figure 1. Structures of detected phytoalexins: **1**, *trans*-resveratrol; **2**, *trans*-arachidin-1; **3**, *trans*-arachidin-2; **4**, *trans*-arachidin-3; **5**, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene; **6**, *trans*-SB-1.

Parts for Peanut Holder. A 3 mL rubber dropper bulb for Pasteur pipets (**Figure 2**, 1) (catalog no. 03-448-26, Fisher) was cut at ca. 25 mm from the round top, and a 10 mm hole (**Figure 2**, 4) was made with a model S-23207 stopper plug cutter (Sargent-Welch Scientific Co., Skokie, IL); a 10 mm spinning puncher/drill was lubricated with glycerol. A 15 × 45 mm clear shell vial (**Figure 2**, 2) (catalog no. 718/61615/00, Alcott Chromatography, Inc., Norcross, GA) and a 11.2-mm OD PharMed tubing (catalog no. A-06484-14, Cole-Parmer) cut to 42–44 mm sections (**Figure 2**, 3) were also used as parts of the holder (**Figure 2**, 6).

Peanut Cultivar. Cultivar Georgia Green was available at the National Peanut Research Laboratory, ARS, USDA, Dawson, GA.

Determination of Maturity Stages. Pods were placed in a wet impact blaster (*17*) to remove the exocarp and expose the color of mesocarp. Pods were separated into four maturity groups based on mesocarp color (yellow, orange, brown, and black) (*18*). Black pods (most mature) were air-dried and stored in a refrigerator at 4 °C for later use in experiments.

Fungal Cultures and Preparation of Spore Inoculate. The following *Aspergillus* cultures were kindly provided by Dr. B. Horn

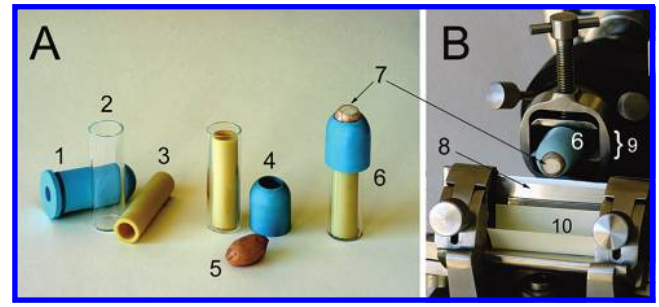

Figure 2. (**A**) Components of peanut holder: 1, rubber pipet bulb; 2, glass vial; 3, rubber tubing; 4, cut bulb 1 with a punched hole; 5, peanut kernel; 6, assembled unit with the peanut top removed with the help of a microtome. (**B**) Fungal spores are applied on surface 7 followed by incubation of the unit; 8, microtome blade; 9, precision horizontal holder that permits installation and reinstallation of unit 6 at 90° against the cutting edge of the blade; 10, folded "Post-it" sticky paper.

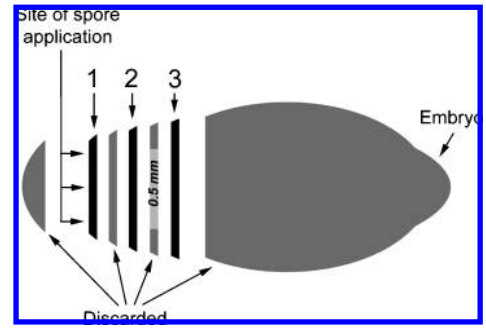

Figure 3. Simplified diagram of a peanut kernel sliced with a microtome after fungal spore application and incubation; 1−3, 0.5 mm thick slices used for analysis.

of the NPRL, ARS, USDA: *A. caelatus* NRRL 25528 (ex type); *A. niger* NRRL 326 (ex type); *A. parasiticus* NPL 32 (wild type, AFL high producer); *A. parasiticus* NPL 33 (white mutant from *A. parasiticus* NPL 32; AFL high producer); *A. parasiticus* NRRL 5862 (wild type); *A. parasiticus* NRRL 6111 (a mutant from *A. parasiticus* NRRL 5862; norsolorinic acid producer); *A. parasiticus* SRRC 163 (a mutant from *A. parasiticus*, NRRL 5862; averantin producer); *A. parasiticus* SRRC 164 (a mutant from *A. parasiticus* NRRL 5862; versicolorin A producer); *A. parasiticus* NRRL 3240 (wild type); *A. parasiticus* SRRC 165 (a mutant from *A. parasiticus* NRRL 3240, averufin producer); *A. parasiticus* NRRL 29602 = NPL P20 (*O*-methylsterigmatocystin producer); *A. flavus* NPL TX 15-2 (AFL high producer, CPA nonproducer); *A. flavus* NPL GA 4-4 (AFL nonproducer, CPA producer); *A. flavus* NRRL 21882 = NPL 45 (AFL nonproducer, CPA nonproducer); and *A. flavus* NRRL 19772 (norsolorinic acid producer). Fungal cultures were grown on Czapek agar slants (*19*) at 30 °C for 14 days. Spores were washed from the slants with water containing Tween 20 (0.1 mL/L) followed by filtration through glass wool and dilution of the spore suspension to the concentration of 1×10^6 spores/mL. Dead spores were obtained from a previous experiment during extraction with $CHCl_3$ large quantities of peanuts inoculated with *A. parasiticus* NRRL 21369 (a UV color mutant of *A. parasiticus* 6111, AFL nonproducer) and incubated for 12 days at 30 °C. Spores, concentrated on the surface of $CHCl_3$, were separated and air-dried. Spore nonvitality was assured by plating on Czapek agar and incubating under favorable conditions.

Plant Material Processing. The procedure for phytoalexin production studies has been described in ref *7*. In brief, blasted, mature, sound peanut pods were surface-sterilized with 2% NaOCl. The seeds were aseptically removed from the hulls and allowed to imbibe a sufficient quantity of water by placing them into sterile distilled water for 18 h at room temperature. The kernels were securely placed in the holder (**Figure 2**, 6) and dissected as shown in **Figure 3**. About one-fifth to one-sixth of each kernel from the side opposite the embryo (**Figure 3**) was removed with a microtome knife (**Figure 2**, 8) forming a 6–8 mm

diameter surface for application of fungal spores (**Figure 2**, 7). A suspension of fungal spores (1×10^6/mL) of one of the above fungal strains was uniformly sprayed over the sliced sides of the kernels. The kernels were then incubated without light at 30 °C and ca. 100% relative humidity for 96 h. Sterile conditions were assured throughout the entire procedure of the plant material preparations. Phytoalexin production was determined in individual 0.5 mm thick slices from the outer wound site (**Figure 3**) after 24, 48, 72, and 96 h of incubation by the following method. Individual slices were ground in an agate mortar with an equal amount (w/w) of Celite 545. The mixture was extracted with 1 mL of MeOH for 30 min with vortexing for 1 min every 5 min. The extract was filtered through a glass fiber filter. From 5 to 50 μL of the filtrate was analyzed by HPLC.

Reference Compounds. *trans*-Resveratrol (**1**) (approximately 99%) was purchased from Sigma. Pure individual peanut stilbenes, *trans*-arachidin-1 (**2**), *trans*-arachidin-2 (**3**), *trans*-arachidin-3 (**4**), *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene (**5**), and SB-1 (**6**) (**Figure 1**), were obtained as described previously (*6, 7*). The identity of resveratrol as well as stilbenes extracted from peanuts was confirmed by ESI-MS/MS and UV spectroscopy. The values determined in this research are given in parenthesis as $[M + H]^+$ 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 (*m/z* 297; 308 and 322 nm), *trans*-arachidin-3 (*m/z* 297; 334 nm), *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene (*m/z* 295; 296 nm), and SB-1 (*m/z* 345; 364 nm). The above results were in agreement with published data (*1–7*).

Standard Solutions of Phytoalexin for HPLC. Stock solutions were prepared by dissolving 0.5−1.5 mg of pure standards in 25 mL of MeOH. Working solutions were prepared daily by diluting aliquots of the stock solutions by the appropriate amount of MeOH to obtain concentrations from 2 to 8 ng/μL.

HPLC-DAD-MS Analyses of Stilbene Phytoalexins. Analyses were performed using an HPLC system equipped with an LC-10ATvp pump (Shimadzu, Kyoto, Japan), an 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 2% HCOOH in H_2O (C) were mixed in the following gradient: initial conditions, 95% A/ 0% B/ 5% C, increased linearly to 0% A/ 95% B/ 5% C in 18 min, held isocratic for 5 min, decreased to initial conditions in 0.01 min. The flow rate was 1.0 mL/min. The column was maintained at 40 °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 500. Heated capillary temperature was 230 °C, sheath gas flow was 45 units, capillary voltage was 8 V, and source voltage was 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* 2.5, 30 or 35%, 0.25, and 30 ms, respectively. Concentrations of *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, and *trans*-SB-1 were determined by reference to peak areas of corresponding pure standards at 317, 339, 322, 334, 296, and 364 nm, 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 a *t* test; the Mann–Whitney rank sum test was applied when the normality test failed ($p < 0.050$).

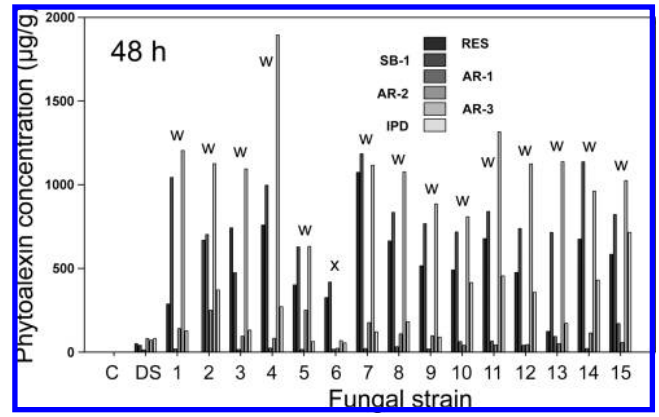

Figure 4. Phytoalexin production by kernels as determined in a single 3 mm thick slice after 48 h of incubation with the following fungal strains: 1, *A. caelatus* NRRL 25528; 2, *A. niger* NRRL 326; 3, *A. parasiticus* NPL 32; 4, *A. parasiticus* NPL 33; 5, *A. flavus* NRRL 21882 = NPL 45; 6, *A. flavus* NPL TX 15-2; 7, *A. flavus* NPL GA 4-4; 8, *A. parasiticus* NRRL 29602 = NPL P20; 9, *A. parasiticus,* NRRL 6111; 10, *A. parasiticus* NRRL 3240; 11, *A. parasiticus* NRRL 5862; 12, *A. parasiticus* SRRC 164; 13, *A. parasiticus* SRRC 163; 14, *A. flavus* NRRL 19772; 15, *A. parasiticus* SRRC 165. Means of total phytoalexin production followed by the same letter are not significantly different, $p = 0.05$, $n = 3$ (for clarity, error bars for the individual phytoalexins are not shown). Abbreviations: RES, *trans*-resveratrol; AR-1, *trans*-arachidin-1; AR-2, *trans*-arachidin-2; AR-3, *trans*-arachidin-3; IPD, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene; C, control (distilled water); DS, dead spores.

RESULTS AND DISCUSSION

The first group of experiments was performed with 15 strains from the genus *Aspergillus*: *A. flavus*, *A. parasiticus*, *A. caelatus*, and *A. niger*. Both *A. flavus* and *A. parasiticus* were represented by natural isolates (AFL and CPA producers and nonproducers) and some mutants that were tested together with their parent strains. The mutants were blocked at different stages of aflatoxin production. The goal of the experiments was to determine possible differences in phytoalexin production by peanut kernels in response to invasion by diverse fungal strains on time basis. The experiments were also intended to develop the experimental setup and optimize the inoculation, incubation, and plant-processing conditions. The main problem concerned the fact that the kernels had to be saturated with water and, therefore, could not be glued or securely positioned by other simple means for microtome dissection after at least 72 h of incubation. After several attempts, a reliable, yet simple device (**Figure 2**) was suggested and successfully used in all experiments. A peanut kernel was gently but firmly positioned between two soft rubber pieces in a glass vial, and the holder with the peanut kernel could be installed before inoculation and then precisely reinstalled after incubation into the microtome holder (**Figure 2**). Such a design allowed for obtaining slices of any appropriate thickness even after 96 h of incubation.

As anticipated, the deviation of the data was considerable due to the need of using different kernels for the same sets of data. One kernel could be used only once to obtain data on the phytoalexin content in three slices at a given time. For the next set of data at a different time another kernel had to be used. However, the data obtained allowed for some conclusions on the phytoalexin accumulation in infected peanut seeds (**Figure 4**). The overall production of phytoalexins elicited by the 15 fungal strains was uniform with the exception of the production elicited by *A. flavus*, NPL TX 15-2 (AFL producer at >100 μg/mL in a liquid medium), which was significantly lower in a

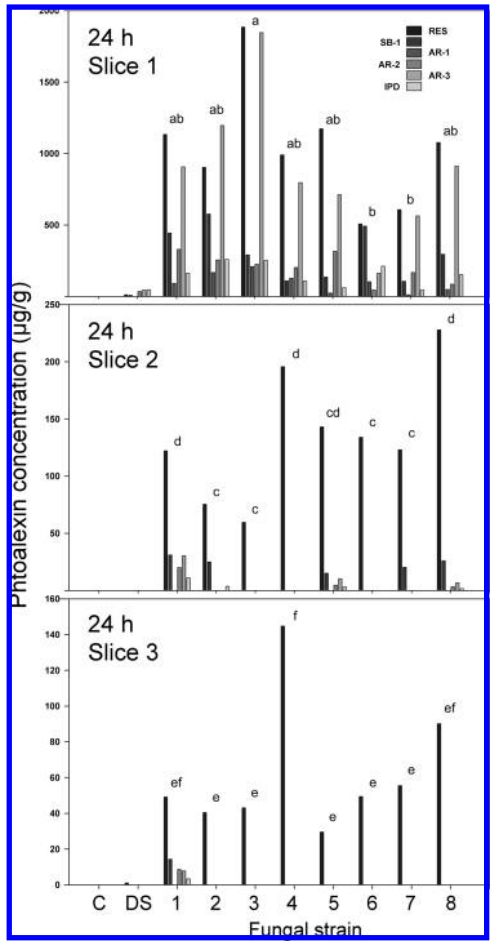

Figure 5. Phytoalexin production by kernels as determined in individual slices (**Figure 3**) after 24 h of incubation with the following fungal strains: 1, *A. caelatus* NRRL 25528; 2, *A. niger* NRRL 326; 3, *A. parasiticus* NPL 32; 4, *A. parasiticus* NPL 33; 5, *A. flavus* NRRL 21882 = NPL 45; 6, *A. flavus* NPL TX 15-2; 7, *A. flavus* NPL GA 4-4; 8, *A. parasiticus* NRRL 29602 = NPL P20. Means of total phytoalexin production followed by the same letter are not significantly different, $p = 0.05$, $n = 3$ (for clarity, error bars for the individual phytoalexins are not shown). Abbreviations: RES, *trans*-resveratrol; AR-1, *trans*-arachidin-1; AR-2, *trans*-arachidin-2; AR-3, *trans*-arachidin-3; IPD, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene; C, control (distilled water); DS, dead spores.

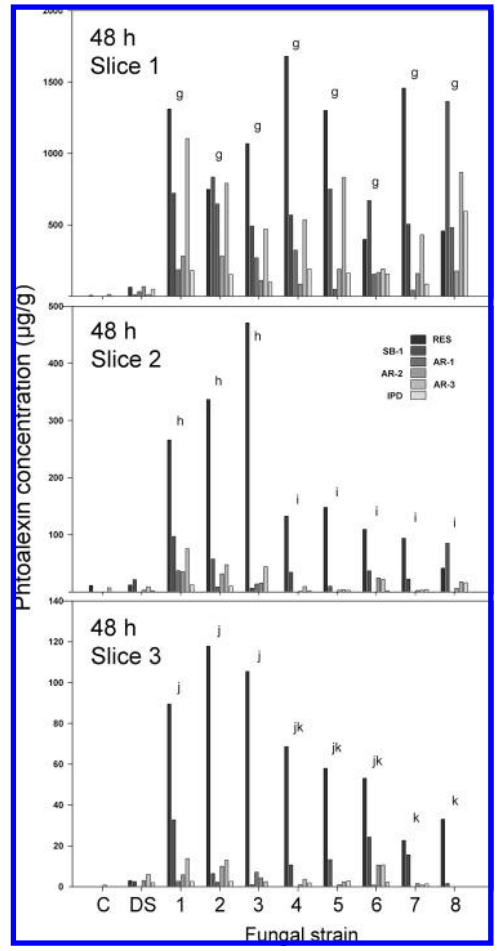

Figure 6. Phytoalexin production by kernels as determined in individual slices (**Figure 3**) after 48 h of incubation with the following fungal strains: 1, *A. caelatus* NRRL 25528; 2, *A. niger* NRRL 326; 3, *A. parasiticus* NPL 32; 4, *A. parasiticus* NPL 33; 5, *A. flavus* NRRL 21882 = NPL 45; 6, *A. flavus* NPL TX 15-2; 7, *A. flavus* NPL GA 4-4; 8, *A. parasiticus* NRRL 29602 = NPL P20. Means of total phytoalexin production followed by the same letter are not significantly different, $p = 0.05$, $n = 3$ (for clarity, error bars for the individual phytoalexins are not shown). Abbreviations: RES, *trans*-resveratrol; AR-1, *trans*-arachidin-1; AR-2, *trans*-arachidin-2; AR-3, *trans*-arachidin-3; IPD, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene; C, control (distilled water); DS, dead spores.

single 3 mm slice after incubation for 48 (**Figure 4**) and 72 h (to reduce the number of illustrations, only the 48 h graph is shown). The toxigenicity of the used strains did not significantly influence the production of phytoalexins. After 24 h of incubation, *trans*-resveratrol was the major metabolite. After 48 h (**Figure 4**), arachidin-3 and SB-1 were the dominant phytoalexins, but after 72 and 96 h, only SB-1 was observed as the major compound with the exception of kernels that were inoculated with *A. niger* NRRL 326 and that were producing *trans*-arachidin-1 (**2**) at significantly higher levels compared with the other strains.

The first eight strains, 1–8 (**Figure 4**), represented a diverse group of isolates that produced less uniform results than the remaining strains and therefore were selected for a second set of experiments (**Figures 5−8**). Dead spores were investigated for their ability to trigger phytoalexin production as well. The degree of peanut kernel involvement (local vs entire kernel) in phytoalexin production was investigated. Individual slices 1–3 (**Figure 3**) were analyzed for the presence of the six phytoalexins that were found to be produced in the first set of experiments with the detection limit of 30 ng/g. To prevent cross-contamination, the two slices between the experimental 1–3 (**Figure 3**) slices were not used for the analysis. Statistical comparison of data represented in **Figures 4−8** was performed within one slice; different individual slices were not statistically compared with each other.

In all of the experiments, data from the control (C) and dead spore (DS) applications (**Figures 4−8**) were significantly different from each other and from the rest of the data; for the purpose of simplicity these significant differences are not reflected in the graphs. *trans*-Resveratrol (**1**) was the major metabolite after 24 h of incubation (**Figure 5**). In slice 1 resveratrol and *trans*-arachidin-3 (**4**) were the major phytoalexins. Apparently, resveratrol is the first stilbenoid that is produced by kernels in the most remote slice 3 (**Figure 5**) because only resveratrol was detected as the major metabolite. In the slices 2 and 3, *A. caelatus* NRRL 25528 caused production of five metabolites with the exception of *trans*-arachidin-1. Two other strains, *A. flavus* NRRL 21882 and *A. parasiticus* NRRL 29602, did not produce arachidin-1 in slice 2 (**Figure 5**). In the first slice, arachidin-1 production was overall significantly lower than that of other phytoalexins. Although

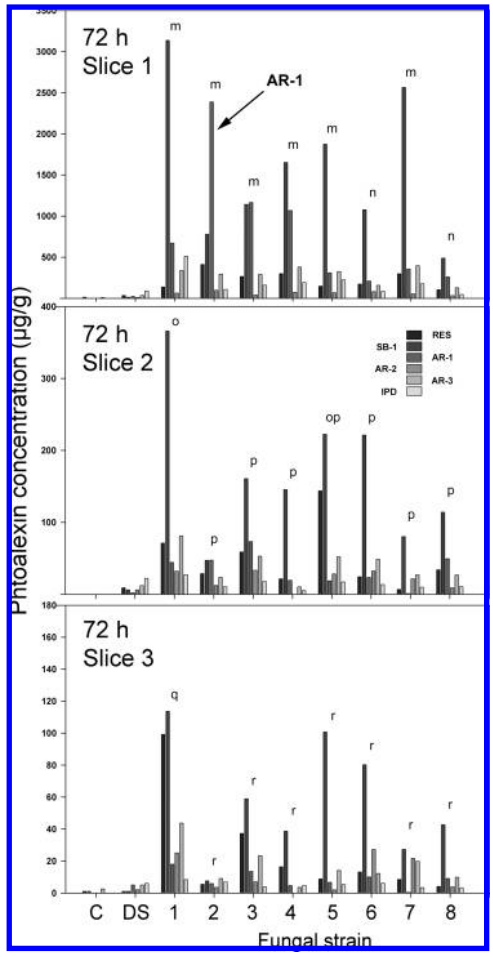

Figure 7. Phytoalexin production by kernels as determined in individual slices (**Figure 3**) after 72 h of incubation with the following fungal strains: 1, *A. caelatus* NRRL 25528; 2, *A. niger* NRRL 326; 3, *A. parasiticus* NPL 32; 4, *A. parasiticus* NPL 33; 5, *A. flavus* NRRL 21882 = NPL 45; 6, *A. flavus* NPL TX 15-2; 7, *A. flavus* NPL GA 4-4; 8, *A. parasiticus* NRRL 29602 = NPL P20. Means of total phytoalexin production followed by the same letter are not significantly different, $p = 0.05$, $n = 3$ (for clarity, error bars for the individual phytoalexins are not shown). Abbreviations: RES, *trans*-resveratrol; AR-1, *trans*-arachidin-1; AR-2, *trans*-arachidin-2; AR-3, *trans*-arachidin-3; IPD, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene; C, control (distilled water); DS, dead spores.

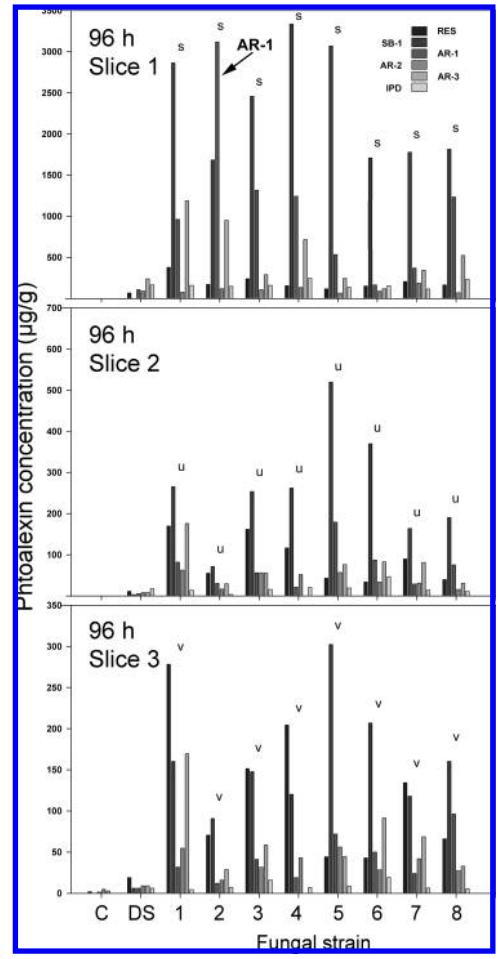

Figure 8. Phytoalexin production by kernels as determined in individual slices (**Figure 3**) after 96 h of incubation with the following fungal strains: 1, *A. caelatus* NRRL 25528; 2, *A. niger* NRRL 326; 3, *A. parasiticus* NPL 32; 4, *A. parasiticus* NPL 33; 5, *A. flavus* NRRL 21882 = NPL 45; 6, *A. flavus* NPL TX 15-2; 7, *A. flavus* NPL GA 4-4; 8, *A. parasiticus* NRRL 29602 = NPL P20. Means of total phytoalexin production followed by the same letter are not significantly different, $p = 0.05$, $n = 3$ (for clarity, error bars for the individual phytoalexins are not shown). Abbreviations: RES, *trans*-resveratrol; AR-1, *trans*-arachidin-1; AR-2, *trans*-arachidin-2; AR-3, *trans*-arachidin-3; IPD, *trans*-3-isopentadienyl-4,3′,5′-trihydroxystilbene; C, control (distilled water); DS, dead spores.

there was a significant difference between the syntheses of phytoalexins caused by some strains (**Figure 5**), no definite correlations could be noted considering fungal toxigenicity as a possible factor influencing the production of phytoalexins.

After 48 h, resveratrol (**1**) remained the major metabolite; however, other phytoalexins were also detected in all slices of all the samples. A significant difference was observed between some samples in slices 2 and 3. However, there was no significant difference between the samples in slice 1 (**Figure 6**). Overall, phytoalexin production in slice 3 was similar to that in a single 3 mm slice (**Figure 4**).

After 72 and 96 h of incubation, resveratrol (**1**) was no longer the major stilbene; a concomitant increase in SB-1 (**6**) concentration was observed. SB-1 became the dominant compound ($p < 0.05$) in all slices of all the samples with one exception, being the sample that was inoculated with *A. niger* NRRL 326, which elicited significantly higher production of *trans*-arachidin-1 (**Figures 7** and **8**) in slice 1, the site of direct contact with the fungus; however, lower layers did not produce any significant concentrations of the stilbene (**Figures 7** and **8**). After 72 h, *A. caelatus* NRRL 25528 stimulated accumulation of SB-1 (**6**) at significantly higher levels than other samples in the two lower layers (**Figure 7**); however, at the 96 h time point the SB-1 content in slices 2 and 3 was not different from that in other inoculated samples. This phenomenon is not understood; there might be some relation with fungal growth rate, a deeper fungal hyphae penetration, or some other, yet undetected, events. Extracts of all slices collected from all of the samples at 96 h did not exhibit any significant difference with the already mentioned exception of the sample inoculated with *A. niger* NRRL 326. No association with fungal toxigenicity after 72 or 96 h of incubation was observed.

Dead fungal spores, even after exhaustive extraction with $CHCl_3$, were able to elicit production of phytoalexins (**Figures 4**−**8**). It is likely that the peanut plant has a genetic ability to recognize chitin or other essential spore compounds that were not extracted with $CHCl_3$.

The ratios of total phytoalexin concentrations in slice 3 versus slice 2 versus slice 1 (**Figure 9**) were 1:2.4:41, 1:3.6:36, 1:2.6:25, and 1:1.1:10 at 24, 48, 72, and 96 h, respectively. These

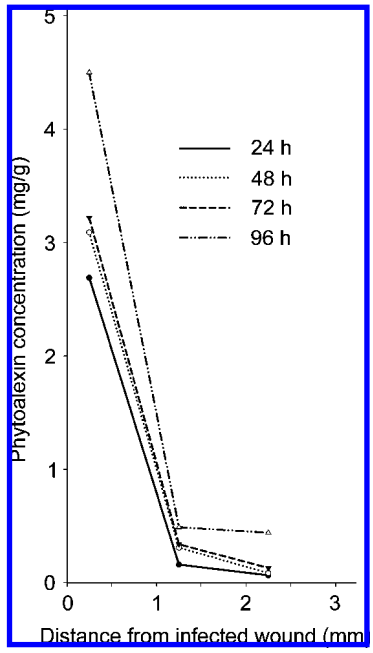

Figure 9. Total average phytoalexin production by inoculated kernels at different depths from infected wounds (**Figure 3**) after 24, 48, 72, and 96 h of incubation.

ratios indicate that with longer incubation times deeper layers of the kernel become more actively involved in the synthesis of phytoalexins. It is also apparent that exceptionally high concentrations of phytoalexins are accumulated at the site of direct contact with the fungus.

Present research demonstrated that different fungal strains from the genus *Aspergillus* were able to elicit intensive phyalexin synthesis in a localized area close to the infected wound of a peanut kernel under conditions favorable to both the host and the fungus. Higher phytoalexin concentrations were accumulated with longer incubation times, and the composition of phytoalexins varied significantly by layer. Resveratrol (**1**) is most likely a building block for other stilbenoids that seem to emerge (almost simultaneously) after resveratrol, with the exception of SB-1 (**6**), which tended to accumulate at longer incubation times. *A. niger* NRRL 326 elicited a significantly different response compared with other tested isolates, causing production of *trans*-arachidin-1. No association was observed between phytoalexin production and toxigenic potential of fungal strains that elicited the production in mature peanut kernels.

ABBREVIATIONS USED

DAD, diode array detector; HPLC, high-performance liquid chromatography; MS, mass spectrometer, mass spectrometry, mass spectra; ESI, electrospray ionization; IPD, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene; AFL, aflatoxins B_1 and B_2 or aflatoxins B_1, B_2, G_1, and G_2; CPA, cyclopiazonic acid.

ACKNOWLEDGMENT

I thank L. Greene for his valuable help in the laboratory and Dr. B. Horn for providing the fungal cultures.

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Received for review December 11, 2007. Revised manuscript received January 11, 2008. Accepted January 15, 2008.

JF703595W