The Effects of Chemical Treatment, Harvest Date, and Specific Isolation Media on the Peanut Shell Mycobiota of Two Peanut Cultivars

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ABSTRACT

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Peanut (*Arachis hypogaea*) cultivars Florunner and Southern Runner grown at two locations near Tifton, Georgia, were either treated with the fungicide flutolanil (Moncut) or nontreated. Comparisons of peanut shell mycobiota were made for both treatments at two harvest dates. A total of 12,744 fungal isolates were cultured from 4,200 shells assayed. Over two-thirds of the isolates were Deuteromycotina. Common form-genera isolated were *Alternaria, Curvularia, Fusarium, Lasiodiplodia, Nigrospora, Rhizoctonia,* and *Rhizopus.* Isolations of some genera were significantly different between treatments within a cultivar, and between the two cultivars. However, these differences were of small magnitude and inconsistent across farms, harvest dates, or cultivars. In particular, *Fusarium, Rhizoctonia,* and *Nigrospora sphaerica* were isolated at significantly different rates from both sites. On Florunner, flutolanil increased total isolations of *Fusarium oxysporum, F. solani,* and *F. equisiti.* Also, several *Rhizoctonia* anastomosis groups (AGs), excluding *R. solani* AG-4, could be obtained more readily from Florunner than from Southern Runner shells. Mean isolations on three media were significantly different. *Rhizoctonia* spp. were isolated from shells at the same frequency with tannic acid-benomyl agar (TABA) (semiselective Basidiomycete medium) and malt extract agar (MEA) media. *Fusarium* spp. were isolated more frequently on malt-salt agar than on MEA and TABA.

The soil microbiota consists of many different microorganisms including bacteria, yeasts, and fungi (13). Pathogenic and saprophytic fungi are major components of soil microbiota which survive on dead plant debris (15). In the peanut (*Arachis hypogaea* L.) agroecosystems, large numbers of pods remain in the soil after harvest (*personal observation*). These shells decay slowly because they are high in lignin (28.7%) and cellulose (48.5%) (9). Unharvested, intact peanut pods have been shown to be a source of inoculum for several soilborne plant pathogens (4).

Pesticides used to protect peanut plants from pathogenic fungi such as *Rhizoctonia solani* Kühn AG-4 (Rhizoctonia limb rot) and *Sclerotium rolfsii* Sacc. (southern stem blight) have been reported to affect the composition of peanut shell mycobiota (3). New fungicides currently are being tested to control peanut stem pathogens *R. solani* anastomosis group (AG) 4 and *S. rolfsii.* Flutolanil (Moncut 50W) is an experimental systemic fungicide which is inhibitory to Basidiomycetes and to a lesser extent to Ascomycetes, Deuteromycotina, and Phycomycetes. This chemical acts by suppressing the production of succinate dehydrogenase involved in fungal respiration (28). The combination of fungal specificity to Basidiomycetes such as *R. solani* and *S. rolfsii,* and chemical systemicity in plants suggests there is the potential of flutolanil to alter the peanut pod and residual shell mycobiota. However, the fungicide is not systemic through the pegs to the pods, and control must occur by direct contact by washing from the foliage and stems to the pod surface.

The chemical composition of peanut pods changes as they mature, thus influencing the suitability of various components as substrates for fungi (32). Hallock et al (17) discussed the concentrations and distributions of P, K, Ca, Mg, Cu, Mn, and Zn during stages of pod maturation. Diener showed that differences in pod age (with resulting differences in mineral and organic compositions) influence colonization by fungi (11). He also demonstrated that the isolation rates of *Aspergillus flavus* Link:Fr. and other agricultural soilborne fungi increase with time and maturity of pods. McDonald (26) observed that *Aspergillus* spp. were more abundant in shells of mature than overmature pods.

Differences in peanut cultivars could have an effect on colonization of shells by various fungi. The cultivar Southern Runner is partially resistant to foliar diseases caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton and *Puccinia arachidis* Speg. (16). Southern Runner was also the most resistant of 16 cultivars evaluated for response to *S. rolfsii,* with about 50% less disease incidence than the commonly grown cultivar Florunner (8). Increased disease resistance of Southern Runner compared to Florunner could indicate differences in the ability of fungi to colonize and survive in pods as well.

As part of an ongoing investigation to identify fungicides which affect peanut pod mycoflora and pathogens harbored in residual peanut shells, this study details the effects of flutolanil on the composition and density of fungal populations in Florunner and Southern Runner shells. Fungi were assayed from half shells at two harvest dates and from two widely separated fields.

MATERIALS AND METHODS

Gibbs farm. Florunner and Southern Runner cultivars were seeded (100 kg/ha) on 11 May 1990 in a field of Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudult; pH 5.8) with a history of continuous peanut culture. Cultural practices and pest controls were conducted according to local cooperative extension service recommendations (23). The field received a total of 10.6, 15.3, 14.3, and 9.9 cm of water in June, July, August, and September, respectively, from rain or irrigation. A split-split plot design with five replications was used. Cultivars were whole plots, digging (harvest) dates were subplots, and fungicide treatments were sub-sub-plots. Each sub-sub-plot consisted of a single bed with two rows (7.62 × 1.83 m) on 0.91-m centers. The test was established in an area known to have high soilborne populations of *S. rolfsii* and *R. solani* AG-4. All plots were oversprayed with a conventional tractor-mounted hydraulic sprayer delivering chlorothalonil (Bravo 720) at the rate of 1.23 kg a.i./ha on a 14-day schedule to control *Cercosporidium personatum* and *Cercospora arachidicola* S. Hori. The fungicide treatments compared in this test were 1) nontreated control, and 2) flutolanil 50WP at 2.24 kg a.i./ha applied by CO_2-powered backpack sprayer in 187 L/ha of water on 6 July and 2 August. Both cultivars were harvested on two dates. Florunner was inverted on 18 September (123 days after planting [DAP]) and 8 October (132 DAP), and harvested on 3 October and 16 October, respectively. Southern Runner was inverted on 27

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September (142 DAP) and 18 October (153 DAP), and harvested on 3 October and 25 October, respectively. The rainfall between digging and harvest was 0.3 cm for the first harvest and 9.6 cm for the second. In the southeastern United States, Florunner requires ca 130–140 days to reach optimum maturity. Southern Runner requires an additional 14–21 days.

Mobay (=Miles) farm. On 9 May 1990, Florunner and Southern Runner cultivars were seeded at a rate of 100 kg/ha in a field of Tifton loamy sand (pH 5.9) under continuous peanut culture and with high populations of *R. solani* and *S. rolfsii.* Recommended practices were followed for routine pest management (23). Chlorothalonil was applied as previously described. Rainfall or irrigation (and average daily temperatures) during the study totaled 10.0 cm (26.7 C) for June, 5.9 cm (28.3 C) for July, 6.9 cm (26.6 C) for August, and 6.5 cm (26.0 C) for September. Data collected between 1925 and 1990 from the Coastal Plain Experiment Station in Tifton, Georgia, showed that normal amounts of rainfall for June, July, August, and September are 11.0, 16.0, 15.2, and 8.5 cm, respectively. Average daily temperatures were 26.0, 27.2, 27.2, and 27.0 C, with an average of 26.9 C over the 4 mo. A split-split-plot design with five replicates was used. Cultivars were whole plots, fungicide treatments were subplots, and harvest dates were sub-sub-plots. Triple-bed, six-row subplots (9.14 $\times$ 5.49 m) with rows on 0.91-m centers were used. Single two-row beds comprised sub-sub-plots that were inverted on 13 September (126 DAP) and 16 October (149 DAP). Plots were combined on 20 September and 31 October, respectively. Rainfall between digging and harvest was 1.3 cm for the first and 3.0 cm for the second. The two treatments used in this test were 1) nontreated control, and 2) flutolanil 50 WP (1.12 kg a.i./ha) applied on 26 July. Flutolanil was applied with a CO_2-powered backpack sprayer with 187 L/ha of water.

Soil moisture records are not available for both tests, although it can be inferred from the data supplied for rain and irrigation. Adequate moisture was maintained at the Gibbs farm, whereas peanuts at the Miles farm were subjected to drought stress. For both tests, peanuts were dried in the field to about 12% (w/w) moisture, harvested with a combine, and stored in mesh bags in a dry storage area. In December 1990, 105 peanut pods per plot from both farms were brought into the laboratory for assay. Both tests were established to meet other objectives as well and did not have the same sub-sub-plot and subplot assignments.

Laboratory procedures. The methodology was the same as that used by Baird et al (2,3), with the exceptions noted below. Shells were placed in petri dishes on the three media described previously, but some ingredients were changed for this study. Malt extract agar (MEA) had dicloran [Botran] added (6 μg/ml) to retard the growth of *Rhizopus arrhizus* A. Fisher; malt-salt agar (MSA) had the NaCl concentration changed from 10 g to 100 g/L. Tannic acid-benomyl agar (TABA) was not changed. Thirty-five shells from each sub-sub-plot (five per 9-cm dish) were plated on each of the three media and incubated at room temperature (ca 22–24 C) for 10 days. No specialized lighting was provided other than the normal fluorescent bulbs used in the laboratory.

Additionally, 60 shells from the Gibbs farm were gathered from plots after the peanuts were inverted but before combining. The pods were separated into three categories. The first had moderate numbers of sclerotia (more than six per shell) on the outer surface; the second had low numbers of sclerotia (one to five per shell); and the third had no visible sclerotia (clean shells) of *S. rolfsii.* The shells were assayed on MEA (five per 9-cm petri dish) to determine 1) whether medium was the limiting factor in the isolation of *S. rolfsii,* and 2) if the lack of sclerotia on shells indicated degree of colonization. Shells from the three groups were processed as described above.

Statistical analyses. Data were recorded as the number of isolates recovered from 35 pods per plot plated in each medium. No attempt was made to catalog particular pod identifications from specific shells. Because total isolates plated always exceeded 35, the number was analyzed as count data. When appropriate, counts were transformed by either square root or log 10 transformation. The data were analyzed for each farm and isolation medium by using the design appropriate to that farm. When means were compared among the media for a farm, the unequal variance *t* test was used to test the three comparisons, and results were recorded in standard mean-separation format (32). When means were the

Table 1. Frequency[x] of fungi isolated from peanut shells by location and harvest date

Fungi	Gibbs farm[y]		Miles farm[z]	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Ascomycetes				
Chaetomium	4	2	2	1
Leptosphaerulina	0	0	<1	0
Neocosmospora	<1	1	<1	<1
Pleospora	0	0	<1	0
Thielavia	0	0	2	2
Basidiomycetes				
Laetisaria	<1	0	<1	<1
Rhizoctonia	19	23	8	21
Sclerotium	<1	<1	<1	<1
Deuteromycotina				
Alternaria	30	14	58	8
Aspergillus	2	<1	8	1
Bipolaris	0	0	<1	0
Cladosporium	0	0	<1	<1
Curvularia	40	23	16	17
Dictyosporium	0	0	<1	0
Epicoccum	1	<1	1	<1
Fusarium	56	58	34	72
Helminthosporium	0	0	<1	0
Humicola	0	<1	<1	0
Lasiodiplodia	6	3	1	1
Myrothecium	<1	<1	<1	<1
Nigrospora	4	5	1	8
Pencillium	<1	<1	1	<1
Phoma	0	<1	<1	<1
Pithomyces	<1	0	<1	0
Stigmella	0	0	<1	<1
Trichoderma	1	2	1	1
Zalerion	0	<1	<1	<1
Oomycetes				
Pythium	0	<1	<1	<1
Zygomycetes				
Choanephora	<1	1	2	0
Cunninghamella	<1	0	<1	0
Mucor	1	2	<1	2
Rhizopus	3	4	5	7
Unknown	0	0	<1	0

[x]% Isolations from total peanut shells per harvest (2,100) at each site. Data represent means from two sites, two fungicide treatments, and three isolation media.

[y]Planted 11 May 1990. Inverted: Florunner—18 September and 8 October 1990; Southern Runner—27 September and 18 October 1990.

[z]Planted 9 May 1990. Inverted: Florunner—13 September and 16 October 1990; Southern Runner—13 September and 16 October 1990.

average of the three media, the standard error of the mean (SEM) was based on the weighted average of the individual medium variance, and the LSD used was computed from the SEM.

RESULTS AND DISCUSSION

Over two-thirds of the 12,744 isolates obtained from peanut shells were Deuteromycotina (Table 1). Basidiomycetes were the second most common group, with the majority being *R. solani* (mainly AG-4), followed by Zygomycetes and Ascomycetes. Results in this study were similar to those of a previous study, where fungi from shells from plants treated with propiconazole (Tilt) were comprised primarily of Fungi Imperfecti and Basidiomycetes (3).

The major genus isolated was *Fusarium,* which comprised approximately 20 and 30% of the isolations from the Miles and Gibbs farms, respectively. Other common genera were *Alternaria, Aspergillus, Curvularia, Lasiodiplodia, Nigrospora,* and *Rhizopus.* Basidiomycetes consisted of *Laetisaria, Rhizoctonia,* and *Sclerotium.* Both multinucleate (AG) and binucleate (CAG) anastomosis groups of *Rhizoctonia* spp. were isolated. Similar groups of fungi were isolated and identified from peanut pods in Virginia (13).

Aspergillus spp., which are important in aflatoxin production, were isolated from 8% or less of shells at each harvest date from both farms. During the 1990 growing season, the weather was extremely hot and dry (refer to Materials and Methods for comparison), and these environmental conditions are known to enhance the growth of *Aspergillus* spp. in peanut pods (5,6,21,30,31). Peanuts at the Miles farm were under much greater drought stress than those at the Gibbs farm and had somewhat higher rates of isolation of *Aspergillus* spp. at the first harvest but not at the second (Table 1). The harvest method employed in the study excluded damaged, shriveled, and empty pods, which are the greatest source of contamination; therefore, the data may not reflect the actual occurrence rate of *Aspergillus* spp. such as *A. flavus.*

The isolation rate of *S. rolfsii* was low in this investigation, although the Gibbs farm had a history of extensive southern blight (8). In this study, however, the genus *Sclerotium* was isolated from less than 1% of the shells from the Gibbs farm, compared with 19–23% for *Rhizoctonia* (Table 1). Although the primary infection court for *S. rolfsii* is peanut stems near the soil surface, the fungus invades peanut pods and occasionally causes extensive pod rot (1,10,22). These pods may be shed from the plant before inverting and would not be present in a sample such as that collected by a combine, further reducing the isolation rates of *S. rolfsii.*

Isolations from pods exhibited moderate numbers of viable sclerotia, resulting in 95% recovery of *S. rolfsii,* with 54% and 2% from the low and clean categories, respectively. Thus, we concluded that when *S. rolfsii* was present on or in peanut shells, the fungus could be readily isolated. Hence, isolation frequencies using MEA probably did reflect the degree of colonization by *S. rolfsii* in pods sampled, but not the overall rates of colonization (i.e., the sampling method underestimated the importance of *S. rolfsii* in pods).

Culture media had a significant impact on isolations of most genera and species isolated (Table 2). In ecological studies on *Rhizoctonia* spp. at this experiment station, the principle medium used to isolate *R. solani* from plant tissue and soil has been TABA (4,34). In the current study, MEA was as effective in isolating *Rhizoctonia* AG or CAG groups. With shells from the Miles and Gibbs farms, the number of isolations was higher for AG-4 plated on MEA than on TABA; but the differences were not significant. *R. solani* AG-2 type 2 was isolated on MEA at higher rates than on TABA with shells collected from both sites. When all *Rhizoctonia* spp. were combined, no differences were seen between MEA and TABA, but rates with MSA were significantly lower with shells from both locations. *Fusarium* spp. were isolated from shells from both farms at significantly higher rates with MSA than with the other two media. These results would be expected, since MSA is usually selective for highly osmophilic fungi such as *Fusarium* and *Aspergillus* spp. Significantly more cultures of *Aspergillus* spp. were isolated on MSA from shells from the Gibbs farm, but MSA was not a significantly better medium than TABA for isolating *Aspergillus* spp. from shells collected at the Miles farm. *Lasiodiplodia* (=*Botryodiplodia*) mean isolations from shells obtained at the Gibbs farm were significantly less on TABA, but there was no difference for shells from the Miles farm. The isolation frequency of *Nigrospora* from shells from both locations was less on TABA than on MEA. Benomyl in TABA may have reduced isolation rates of the two genera.

All pathogenic and saprophytic fungi

Table 2. Number of isolations on MEA, MSA, and TABA[x] of pathogenic and most common saprophytic fungi isolated from peanut shells at two locations[y]

	Gibbs farm						Miles farm					
Fungi represented	**MEA**	**(SE)**	**MSA**	**(SE)**	**TABA**	**(SE)**	**MEA**	**(SE)**	**MSA**	**(SE)**	**TABA**	**(SE)**
Alternaria alternata (Fr.:Fr.) Keissl.	8.6a[z]	(1.5)	3.9b	(0.8)	11.3a	(1.9)	7.1b	(1.4)	10.7b	(2.0)	16.9a	(2.4)
Aspergillus flavus Link:Fr.	<1	(<1)	1.1d	(0.3)	0.1e	(<1)	0.2b	(0.1)	0.7a	(0.2)	1.3a	(0.4)
A. niger Tiegh.	0.1e	(0.1)	0.3d	(0.1)	0.1e	(<1)	0.1e	(0.1)	0.2e	(0.1)	1.4d	(0.4)
Curvularia lunata (Wakk.) Boedijn	8.9b	(1.4)	9.1b	(1.5)	15.5a	(1.8)	3.9e	(0.7)	2.9e	(0.5)	10.6d	(1.6)
Fusarium equiseti (Corda) Sacc.	3.8b	(0.6)	7.0a	(1.0)	3.8b	(0.9)	3.4ab	(0.8)	5.1a	(0.8)	2.9b	(0.7)
F. oxysporum Schlechtend.:Fr.	4.3d	(0.6)	2.8e	(0.5)	2.7e	(0.5)	3.9g	(0.7)	1.8h	(0.7)	2.6g	(0.5)
F. solani (Mart.) Sacc.	8.5b	(1.3)	15.3a	(2.0)	10.3b	(1.7)	6.6b	(0.9)	14.0a	(1.4)	11.6a	(1.6)
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	2.0d	(0.4)	1.9d	(0.4)	0.6e	(0.2)	0.4a	(0.1)	0.6a	(0.2)	0.4a	(0.1)
Nigrospora sphaerica (Sacc.) E. Mason	3.7d	(0.7)	0.8e	(0.2)	0.5e	(0.2)	3.1a	(0.6)	1.3b	(0.4)	0.4c	(0.2)
Rhizopus arrhizus A. Fischer	1.4d	(0.3)	0.1e	(<1)	1.4d	(0.4)	1.4d	(0.3)	0.1e	(0.1)	2.0d	(0.4)
Rhizoctonia spp. AG-2-2	2.0d	(0.5)	0.1f	(0.1)	0.4e	(0.1)	0.9g	(0.2)	0.0h	(...)	0.4g	(0.1)
AG-4	10.4g	(1.6)	0.0h	(...)	8.6g	(1.5)	7.1g	(1.2)	0.1h	(0.1)	5.3g	(0.8)
All *Aspergillus* spp.	0.1e	(0.1)	1.4d	(0.4)	0.2e	(0.1)	0.3e	(0.1)	1.4d	(0.3)	2.6d	(0.8)
All *Fusarium* spp.	12.9b	(1.8)	22.6a	(2.7)	14.7b	(2.5)	10.9e	(1.5)	20.1d	(2.1)	16.3d	(2.2)
All *Rhizopus spp.*	1.8d	(0.4)	0.2e	(0.1)	1.9d	(0.5)	2.0b	(0.4)	0.1c	(0.1)	3.9a	(0.9)
All Rhizoctonia spp.	13.4g	(1.9)	0.1h	(0.1)	9.2g	(1.5)	8.4g	(1.3)	0.1h	(0.1)	6.5g	(1.0)
All fungi	60.9a	(6.5)	44.6b	(4.6)	61.4a	(6.4)	44.3b	(4.1)	40.5b	(2.8)	66.9a	(6.1)

[x] MEA = malt extract agar; MSA = malt-salt agar; and TABA = tannic acid-benomyl agar.

[y] From 35 peanut shells for each medium averaged across two harvest dates, flutolanil treated and nontreated, and peanut cultivars Florunner and Southern Runner.

[z] Means within a row for each location followed by same letter or no letter are not different at $P < 0.05$ according to pairwise unequal variance t tests. The letters a–c are from the actual data, d–f from square root transformed data, and g–h from log transformed data.

Table 3. Cultivar differences by treatment and treatment differences by cultivar in number of isolations of the most common fungi and of all fungi obtained from peanut shells at two harvests at each of two locations[x]

Fungus	Harvest 1			Harvest 2			
	Flutolanil	Nontreated	Dif.	Flutolanil	Nontreated	Dif.	LSD (0.05)[y]
Fusarium solani							
Gibbs farm							
Florunner	7.0	11.0	−4.1	10.0	6.0	+4.4*[z]	
Southern Runner	16.0	15.0	+1.3	12.0	13.0	−1.1	7.9
Dif.	−9.7*	−4.4		−1.9	−7.4		
Miles farm							
Florunner	8.0	8.0	−0.1	13.0	12.0	+1.3	
Southern Runner	9.0	6.0	+3.3	17.0	13.0	+3.8	6.4
Dif.	−1.2	+2.1		−3.7	−1.3		
All *Fusarium* spp.							
Gibbs farm							
Florunner	10.0	14.0	−3.8	17.0	11.0	+6.3	
Southern Runner	23.0	20.0	+2.5	19.0	20.0	−1.6	11.1
Dif.	−13.1*	−6.8		−1.8	−9.7		
Miles farm							
Florunner	10.0	11.0	−0.6	21.0	18.0	+2.9	
Southern Runner	11.0	8.0	+2.1	25.0	21.0	+4.1	8.5
Dif.	−0.3	+2.5		−4.4	−3.2		
Rhizoctonia solani AG-4							
Gibbs farm							
Florunner	2.0	7.0	−4.8	7.0	9.0	−2.1	
Southern Runner	6.0	9.0	−2.5	3.0	8.0	−4.3	6.6
Dif.	−4.1	−1.8		+3.3	+1.1		
Miles farm							
Florunner	2.0	3.0	−1.6	8.0	7.0	+0.9	
Southern Runner	1.0	2.0	−0.9	5.0	6.0	−0.8	4.1
Dif.	+0.7	+1.3		+2.7	+0.9		
All *Rhizoctonia* spp.							
Gibbs farm							
Florunner	2.0	8.0	−5.2	9.0	11.0	−2.5	
Southern Runner	7.0	10.0	−2.8	4.0	9.0	−5.0	7.4
Dif.	−4.5	−2.1		+4.7	−2.2		
Miles farm							
Florunner	3.0	4.0	−1.2	9.0	8.0	+1.7	
Southern Runner	1.0	2.0	−1.0	6.0	6.0	−0.7	4.8
Dif.	+1.7	+2.0		+3.7	+1.3		
Aspergillus spp.							
Gibbs farm							
Florunner	<1.0	1.0	−0.9	<1.0	<1.0	+0.4*	
Southern Runner	1.0	1.0	−0.6	<1.0	<1.0	+0.1	1.1
Dif.	−0.5	−0.2		+0.2	+0.1		
Miles farm							
Florunner	3.0	4.0	−0.5	<1.0	<1.0	−0.2	
Southern Runner	2.0	2.0	+0.3	<1.0	<1.0	−0.1	2.2
Dif.	+1.0	+1.9		+0.1	+0.3		
Lasiodiplodia theabromae							
Gibbs farm							
Florunner	1.0	1.0	+0.4	1.0	1.0	−0.1	
Southern Runner	4.0	2.0	+1.2	<1.0	<1.0	+0.3	1.5
Dif.	−2.4*	−1.6*		+0.5	+0.8		
Miles farm							
Florunner	0.3	0.6	−0.3	0.7	0.6	+0.1	
Southern Runner	0.6	0.3	+0.3	0.4	0.1	+0.3	0.6
Dif.	−0.3	+0.3*		+0.3	+0.5		
All *Rhizopus* spp.							
Gibbs farm							
Florunner	1.0	1.0	0	2.0	1.0	+1.1	
Southern Runner	2.0	1.0	+1.6	2.0	1.0	+1.5	1.7
Dif.	−1.7*	−0.1		−0.5	−0.1		
Miles farm							
Florunner	2.0	2.0	+0.5	2.0	1.0	+1.1	
Southern Runner	3.0	<1.0	+2.1	2.0	4.0	−1.8*	2.7
Dif.	−0.3	+1.3		+0.5	−2.4*		
All fungi							
Gibbs farm							
Florunner	52.0	62.0	−9.3	57.0	45.0	+12.2	
Southern Runner	65.0	62.0	+3.0	51.0	51.0	−0.4	28.0
Dif.	−12.7	−0.3		+6.5	−6.1		
Miles farm							
Florunner	52.0	56.0	−4.3	52.0	46.0	+6.6	
Southern Runner	57.0	37.0	−20.1	53.0	50.0	+2.9*	22.5
Dif.	−5.2	19.3*		+0.7	+4.4		

[x]Number of isolations from 35 peanut shells for each value averaged across three isolation media and five replications.
[y]Calculated from the weighted average of the variance for each of the isolation media.
[z]* = Significant difference ($P < 0.05$) by analysis of variance (ANOVA).

isolated more than 50 times from both field tests were analyzed to compare the effect of flutolanil on shell mycobiota mean isolations by cultivar. Isolation frequencies from shells from both farms were significantly different for *Fusarium, Rhizoctonia,* and *Nigrospora sphaerica* (Sacc.) E. Mason (Table 3), but no differences were seen for *Aspergillus spp.* When isolation frequencies of *Aspergillus niger* Tiegh. from the Gibbs farm shells were analyzed by harvest, a higher number of isolations was observed for Southern Runner than for Florunner.

Analyses for *Fusarium* spp. showed the largest numbers of significant differences (Table 3). Significantly more isolations of *Fusarium solani* (Mart.) Sacc. were obtained at harvest 1 from Southern Runner than from Florunner treated with flutolanil. At harvest 2, signficantly greater isolations were observed for Florunner shells treated with flutolanil. When all *Fusarium* spp. were evaluated together, no differences occurred. Not shown in Table 3, the flutolanil treatment increased the number of isolations (+2.6) of *Fusarium oxysporum* Schlechtend.:Fr. from Florunner shells. Subspecies of *F. oxysporum* and *F. solani* are pathogenic to peanut as well as numerous other crops (7,24,27). If the rise in *Fusarium* isolations with flutolanil results in increased inoculum, subsequent susceptible cross planting in these sites could be affected.

The *Pythium–Fusarium* pod rot complex, which plays a significant role in the decay of shells, may have influenced the levels of *Fusarium* spp. present in this study (12). Researchers have shown that *Fusarium* spp. predispose the peanut pods to infection by *Pythium myriotylum* Drechs. (14). After further decay and cellulose depletion by *P. myriotylum, F. solani* and other saprophytic organisms cause disintegration of pods (shells). Garren (14) determined, however, that *F. solani* can become the primary pathogen and cause pod rot when temperatures range between 20 and 28 C. This temperature range is considerably lower than the optimum of 32 C for *P. myriotylum.* Also, *F. oxysporum* and *Fusarium sambucinum* Funkel are potentially pathogenic to peanut seedlings, pods, and mature plants. This could be especially significant in the peanut pod rot complex, which is usually related to nutritional imbalances (29). In this study, very little pod rot other than that obviously associated with *S. rolfsii* was observed in the test fields, and therefore no evaluation was made.

When all *Rhizoctonia* spp. were evaluated together and individually, no significant differences occurred (Table 3). Contrary to this finding, flutolanil was previously shown to significantly decrease the incidence of *R. solani* AG-4 (2). In an earlier study, four treatments of propiconazole were compared for their effects on peanut shell mycobiota, and no significant differences were seen in the number of isolations of *R. solani* AGs (3).

The most common fungi isolated were *Alternaria alternata* (Fr.:Fr.) Keissl., *Aspergillus* spp., *Curvularia lunata* (Wakk.) Boedijn, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Nigrospora sphaerica,* and all *Rhizopus* spp. As shown in Table 3, several differences were found due to peanut cultivar or flutolanil usage for *Aspergillus* spp., *L. theobromae, Rhizopus* spp., and all fungi combined. The differences, however, do not show a pattern when comparing treatments, harvest dates, or cultivars.

Many of the fungi observed in this study were also found on and in parts of peanut pods by previous researchers (18–21,25,26). With a few exceptions, isolations of two *Fusarium* spp. increased; and there was a trend toward reduced isolations of *Rhizoctonia* spp. when flutolanil was used. Overall, flutolanil applied to foliage had limited influence on the composition of the peanut shell mycobiota even though it is very effective on soilborne peanut pathogens. The fungicide is systemic but is not transported down through the pegs and into the pods. The chemical is washed from the foliage and goes into the soil and directly contacts the pods in the geocarposphere. If insufficient amounts of the chemical come in contact with the pods, the effect on the pod mycobiota is reduced. In this investigation, we hypothesized that minimal contact occurred between the fungicide and the pods growing in the soil.

Pods left in the soil may be diseased and include a more diverse mycobiota than do the combined pods analyzed in this study. In a later investigation, *Rhizoctonia* AG and CAG groups, *Fusarium, Mucor,* and *Trichoderma* spp. were significantly greater in detached pods (2). Conversely, isolations of *Alternaria, Curvularia, Nigrospora,* and *Phoma* spp. were lower on detached versus mechanically harvested pods.

A previous study on Florunner peanut demonstrated that 630–778 kg/ha of peanut pods could be left in the soil after harvest (33). This represents a large reservoir of inoculum for soilborne pathogens and aflatoxin-producing fungi. Therefore, any factors influencing fungal colonization of pods, such as those demonstrated in this study, could alter inoculum levels for susceptible crops immediately following peanut.

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