The Peanut Shell Mycobiota of Detached vs. Mechanically Harvested Pods Either Treated or Not Treated with Flutolanil

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ABSTRACT

Baird, R. E., Brenneman, T. B., Bell, D. K., Culbreath, A. K., and Mullinix, B. G. 1993. The peanut shell mycobiota of detached vs. mechanically harvested pods either treated or not treated with flutolanil. Plant Dis. 77:405-408.

Flutolanil (Moncut) was applied to peanut (*Arachis hypogaea*) cv. Florunner at a single rate of 2.24 kg a.i./ha at four locations. Peanut pods from treated and control plots were harvested mechanically by combine, and mycoflora from these shells were compared with mycoflora from shells of detached pods removed from the soil 28–35 days after harvesting. Shells were plated in the laboratory, and over 85% of the 6,648 cultures of fungi obtained were Deuteromycetes. The nine most common genera, with >100 isolations, were *Alternaria, Curvularia, Fusarium, Lasiodiplodia, Mucor, Nigrospora, Phoma, Rhizoctonia*, and *Trichoderma. Fusarium* spp. were isolated most frequently and comprised 42% of the total cultures. Flutolanil treatment significantly decreased the incidences of *Rhizoctonia solani* AG-4 and *Trichoderma* spp. Incidences of other potential pathogens, such as *Fusarium solani* and *F. oxysporum*, were higher in the flutolanil-treated plots than in the control plots. Colonization by *Rhizoctonia* AG and CAG, *Fusarium, Mucor*, and *Trichoderma* spp. was significantly greater in shells from detached pods. Isolation frequencies of *Alternaria, Curvularia, Nigrospora*, and *Phoma* spp., however, were lower from shells of detached pods than from shells of mechanically harvested pods. Isolation frequencies of the mycotoxin-producing fungi *Aspergillus* spp. were low for both mechanically harvested and combined shells.

Information on peanut shell (geocarp) mycobiota is limited, and there are few reports of the effects of pesticides on seed and shell mycobiota when applied to peanut (*Arachis hypogaea* L.). The four existing studies consisted of assaying fungi in whole pods or seed. We have observed, however, that peanut shells may remain intact in soils long after the seed have completely decomposed. In two previous investigations, the fungicides propiconazole (Tilt) (2) and flutolanil (Moncut 50W) (R. E. Baird et al, *unpublished*) were evaluated for effects on the mycobiota from shells from mechanically harvested pods. The results obtained from the propiconazole study (2) showed few differences in isolation frequencies of genera and species. In the flutolanil investigation (R. E. Baird et al, *unpublished*), however, isolation frequencies of several genera were affected by treatments within a cultivar and between cultivars, by two harvest dates, and by comparisons between isolation media. These data, however, were obtained from mechanically harvested shells and may not reflect the actual levels of pathogenic fungi present in the fields.

Accepted for publication 8 December 1992.

In prior investigations (5–8), fungi were obtained from pods either mechanically harvested by combine or attached to hand-pulled plants. Almost no studies were available comparing fungal populations from shells of mechanically harvested pods vs. shells from pods removed by hand from the soil after harvesting. Detached pods can be defined as those left in the soil during digging and combining or because of disease to the pegs. We hypothesized that the percentage of pathogenic fungi may be higher in the shells of pods left in the ground because of pegs weakened or destroyed by fungi and insects. If our hypothesis is correct, data obtained from shells of mechanically harvested pods may underestimate the actual diversity and density of soil mycobiota that are important in understanding inoculum reservoirs and disease control. Unharvested detached pods may serve as a source of inoculum of soilborne plant pathogens (2; R. E. Baird et al, *unpublished*). However, population diversities and densities for pathogens associated with pods have not been characterized.

This paper is the second in a series to quantify the effects of fungicides on peanut shell mycobiota. The purpose of this investigation was to determine 1) the effects of flutolanil on fungal species in shells from pods from flutolanil-treated vs. nontreated peanut plants and 2) if numbers and taxa of fungi isolated from shells of mechanically harvested pods can be correlated with similar data obtained from combined shells vs. detached (hand-harvested) pods left in the soil after harvesting.

MATERIALS AND METHODS

Experimental design and field collections. In May 1991, four trials were conducted at the Blackshank and Gibbs farms (two trials per farm) at the University of Georgia Coastal Plain Experiment Station at Tifton. Soils in all locations were heavily infested with natural populations of multinucleate *Rhizoctonia solani* Kühn AG-4, binucleate (CAG) *Rhizoctonia* spp., *Sclerotium rolfsii* Sacc., and *Fusarium* spp.

At the Blackshank farm, field A had been planted to peanut in 1989 and 1990 and field B had been planted to soybean (*Glycine max* (L). Merr.) in 1989 and to peanut in 1990. Standard cultural and pest control practices for peanut were followed using those previously defined for Georgia (9). On 15 May, peanut cv. Florunner was planted at a seed rate of 100 kg/ha into one two-row plot 7.5 × 1.8 m at field A and one two-row plot 9.1 × 1.8 m at field B. At both sites, rainfall and irrigation during July, August, and September totaled 24.5, 17.0, and 16.3 cm, respectively. The soil type at both sites was a Fuquay sand (0–5% slope, Arenic Plinthic Paleaquult;

pH 6.7). A split-plot experiment with a randomized complete block design was used for both sites, with four replicates per treatment. Whole-plot treatments consisted of the nontreated control and two applications of 2.24 kg a.i./ha of flutolanil, the first on 11 July (57 days after planting [DAP]) and 18 August (95 DAP). Flutolanil was applied with a CO_2-pressurized backpack sprayer delivering 187 L/ha of water at 30 psi. Plants in all plots were over-sprayed, via a conventional tractor-mounted hydraulic sprayer, with chlorothalonil (Bravo 720, 1.23 kg a.i./ha) on a 14-day schedule to control late leaf spot caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton. All peanut plants at the Blackshank farm were mechanically inverted on 25 September and combined on 8 October. Detached pods were removed from the uppermost 25 cm of soil by hand on 12 November.

Both sites at the Gibbs farm, designated fields C and D, had a history of continuous peanut production and were seeded with Florunner at 100 kg/ha on 17 May 1991. Soil type in both fields was Tifton loamy sand (fine-loamy, siliceous, Thermic Plinthic Paleudult; pH 6.4). Except for fungicide applications, recommended practices were followed for cultural and pest management (9). At both sites, rainfall and irrigation during May, June, July, August, and September totaled 17.1, 13.9, 26.8, 7.4, and 10.0 cm, respectively. In both fields, a replicate consisted of one two-row plot 7.6 × 1.8 m. Chlorothalonil was applied as previously described for the Blackshank farm trials. Treatments were as described previously except that flutolanil was applied on 14 July (63 DAP) and 14 August (94 DAP). Plants were dug and inverted on 15 October and combined 24 October. Detached peanut pods were removed from the soil on 12 November as described previously.

All mechanically harvested peanut pods were dried in a crop drier (35 C) to approximately 12% (w/w) moisture and stored in mesh bags in a dry area. These pods were randomly selected from the bags and their shell condition was clean, with few obvious surface infections. All residual peanut pods removed from the soil by hand were dried in the laboratory to approximately 12% (w/w) moisture. The majority of these pods had various levels of surface infections, and the shell tissue was often damaged. In December 1991, half shells from 80 pods per replicate from each location were assayed for mycobiota diversity in the laboratory.

Laboratory procedures. Methodology was the same as that used in the study with propiconazole (2) with the following exceptions. The half shells were placed in petri plates (9 cm diameter) on two types of media: malt-extract agar (MEA) with 6 μg/L of dicloran (Botran) and malt-salt agar (MSA). Forty half shells from each replicate were plated, four per dish, onto each medium and incubated at room temperature (approximately 22–24 C) for 10 days. All fungi growing from shells were subcultured and plated onto potato-dextrose agar medium for identification. Frequency of isolation was determined for the different fungi obtained as the percentage of total isolations.

Procedures for statistical analysis. Analysis of variance was used to evaluate effects of location, fungicide, and pod source on isolation frequency of the various fungi (11). The Waller-Duncan *k*-ratio *t* test was used to perform mean separation ($P \leq 0.05$) for fungi isolated 100 or more times.

Table 1. An overall summary of fungi isolated from shells of peanut cv. Florunner collected from field plots in 1991

Fungi	Isolation frequency[a] (%)
Ascomycetes	
Chaetomium	<1
Neocosmospora	<1
Pleospora	<1
Thielavia	<1
Basidiomycetes	
Laetisaria	<1
Rhizoctonia	10.1
Sclerotium	<1
Deuteromycetes	
Alternaria	22.9
Aspergillus	<1
Bipolaris	<1
Cladosporium	1.0
Curvularia	6.2
Cylindrocladium	1.4
Didymosporium	<1
Diheterospora	<1
Epicoccum	<1
Fusarium	44.4
Gliocladium	2.5
Humicola	<1
Idriella	<1
Lasiodiplodia	2.5
Nigrospora	14.9
Papulaspora	<1
Penicillium	<1
Phoma	6.0
Pithomyces	<1
Sphaeropsis	1.5
Stemphylium	<1
Stigmella	<1
Trichoderma	7.0
Oomycetes	
Pythium	1.1
Zygomycetes	
Cunninghamella	<1
Mucor	2.9
Rhizopus	1.1
Other fungi	4.0

[a] Data are the percentage of 5,120 shells from two field trials each having two locations. Included were equal numbers of shells plated on malt-extract and malt-salt agar and shells treated and not treated with flutolanil at 2.24 kg a.i./ha. Percentages total more than 100 because some shells had more than one fungus.

RESULTS AND DISCUSSION

Of the 6,648 isolates of fungi obtained from peanut shells, over 80% were Deuteromycetes (Table 1). The second most common group of fungi were the Basidiomycetes, with *R. solani* AG-4 the most prevalent, followed by Zygomycetes and Ascomycetes. The results were similar to those in previous investigations with propiconazole (2) and flutolanil (R. E. Baird et al, *unpublished*), in which over 66% of the peanut shell isolations were from the Deuteromycetes.

Fusarium spp. were the most abundant fungi isolated from shells (42%), and *F. equiseti* (Corda) Sacc. was the most common species. Previous investigations (2,5–7; R. E. Baird et al, *unpublished*) showed similar results with *Fusarium* spp. as common inhabitants of peanut shells. Other frequentiy isolated genera (>100 isolates each) included *Alternaria, Curvularia, Lasiodiplodia, Mucor, Nigrospora, Phoma, Rhizoctonia*, and *Trichoderma*. Garren (4) reported that *Fusarium, Phoma, Rhizoctonia*, and *Trichoderma* spp. were common components of the peanut pod mycobiota. In this study, genera of Basidiomycetes isolated from peanut shells included *Rhizoctonia, Sclerotium*, and *Laetisaria*. The *Rhizoctonia* spp. included *R. solani* AG-4, two other multinucleate AGs, and two binucleate CAGs. In 1991, frequencies of *S. rolfsii* were low even though the four locations were known to have a high incidence of such infection among the peanut plants (R. E. Baird et al, *unpublished*). Although *S. rolfsii* often can be isolated from peanut seed and shells from plants in disease loci, the fungus is not a frequent component of the peanut pod mycoflora (4,7). This indicates that *S. rolfsii* survives primarily as sclerotia or in other colonized plant material (1). The third Basidiomycete, *Laetisaria arvalis* Burdsall, also had low numbers of isolations from the Blackshank farm trials.

In the analysis of the effects of flutolanil treatment and shell source on mycobiota isolated more than 100 times from peanut shells in the four trials (Table 2), the important peanut pathogen *Cylindrocladium* was included even though it was isolated fewer than 100 times; no differences were observed among treatments, however. Isolation frequencies for most of the other genera were significantly different ($P \leq 0.05$) when shells from combine-harvested vs. residual pods and flutolanil treatments were evaluated (Table 2). The isolation frequencies for *Nigrospora* were not significantly different for harvested vs. residual pods, but *Rhizoctonia* was isolated more frequently from residual peanut pods than from those mechanically harvested.

Within chemical treatments, shell source was significant for *Alternaria,*

Curvularia, Fusarium, Mucor, Nigrospora, and *Phoma*, with higher isolation rates from shells treated with flutolanil. The flutolanil treatment significantly reduced isolation rates for *Rhizoctonia* and *Trichoderma* spp., and residual peanut shells had significantly higher rates than mechanically harvested pods. Flutolanil was inhibitory to *Trichoderma* spp. (Table 2), which may hinder the effectiveness of these fungi as natural biological control agents of pathogens such as *R. solani* AG-4 and AG-2-2 and *S. rolfsii*. With shells from flutolanil-treated plants, the isolation frequencies for *Rhizoctonia* were significantly lower at all four locations.

Analysis of the *R. solani* anastomosis groups observed in the study (Table 3) showed that the trend for *R. solani* AG-4 was similar to that for *Rhizoctonia* spp. (Table 2), with significantly lower isolation rates from shells from flutolanil-treated plants than from nontreated plants. Minor treatment differences were observed for *R. solani* AG-2-2 from residual shells where flutolanil treatment resulted in decreased isolation frequencies. Data from Tables 2 and 3 indicate that the presence or absence of a vital contact with the parent plant may have influenced isolation frequencies of the fungi. Therefore, results from previous mycoflora assays in which pods were attached to plants after inverting may not have given an accurate estimate of the amount of inoculum of *R. solani* AG-4 in residual shells left in the soil after harvest.

The isolation rates for *F. solani* were significantly different between treatments when *Fusarium* spp. were evaluated separately (Table 3). The frequencies of *F. solani* were higher with flutolanil treatments when shells from both attached and residual pods were evaluated. Also, *F. equiseti* was isolated more frequently from mechanically harvested (attached) pods than from residual pods. No significant interactions were observed between pod source and treatments for specific *Rhizoctonia* or *Fusarium* taxa. In our previous investigation with flutolanil (R. E. Baird et al, *unpublished*), isolation frequencies of *Fusarium* spp., including *F. solani*, were increased with flutolanil treatment. Numerous species and subspecies of *Fusarium* are pathogenic to agricultural crops (12–14), and flutolanil may enhance the pathogenic species of *Fusarium*, which could result in increased disease incidence in susceptible crops.

The effect of media on isolation frequencies was significant for *Rhizoctonia* (MEA, 7.5%; MSA, 2.4%) and *Trichoderma* spp. (MEA, 3.8%; MSA, 7.9%). Species from both genera had significantly greater isolation frequencies on MEA, whereas *F. solani* (MEA, 6%; MSA, 7.9%), *F. equiseti* (MEA, 5.7%; MSA, 10%), and *Alternaria* spp. (MEA, 10%; MSA, 12.7%) were isolated more commonly on MSA. Previous studies (1) showed that MEA was an excellent medium for isolation of both *Rhizoctonia* AG and CAG groups; *Fusarium* and *Alternaria* spp. also were significantly greater on MSA than on MEA.

The majority of the 34 genera of fungi isolated during this study had been identified in previous investigations without fungicides (5–7), but this study demonstrated that isolation frequencies of fungi were altered by flutolanil treatment. Flutolanil significantly reduced isolation frequencies of *Rhizoctonia* spp., particularly AG-4, and increased isolation frequencies of *F. solani* and *F. oxysporum*. Shells from residual pods had significantly greater levels of *Rhizoctonia* and *Fusarium* than did shells from mechanically harvested attached pods. These findings indicate that previous investigations studying peanut shell mycobiota may have been incomplete in assaying the diversity and density of pod-borne fungi. These results also indicate that flutolanil can reduce the incidence of *R. solani* AG-4 in residual peanut shells and thereby aid in controlling the pathogen in susceptible crops immediately following peanut.

Detached peanut pods left in the soil after harvest can be a source of inoculum for some plant pathogens. The number of pods left in the soil, however, depends on previous disease pressure, environmental conditions, and the state of crop maturity when harvested (10). Management practices that reduce either the number of pods in soil or the colonization of or survival on those pods by pathogens may be beneficial in reducing inoculum levels for future crops. The role of flutolanil in such a strategy should be investigated for various crops susceptible to *R. solani* AG-4 grown in rotation with peanut.

Table 2. Effects of pod shell source and flutolanil treatment on isolation frequencies of common peanut shell mycobiota

	Detached pods		Harvested pods	
Genera[a]	Treated	Untreated	Treated	Untreated
Alternaria‡	20.55*+[b]	8.75*+	31.80*	30.70*
Curvularia‡	5.48*+	1.80*+	8.90*	8.53*
Cylindrocladium	1.48	1.95	1.48	0.78
Fusarium	53.04*+	43.85*+	43.75*+	37.02*+
Lasiodiplodia	0.60	0.48	0.50	0.49
Mucor	4.83*	2.73*	2.20*	1.65*
Nigrospora	15.78+	11.25+	17.83+	14.70+
Phoma	2.50*+	0.95*+	11.10*+	9.30*+
Rhizoctonia	11.13*+	15.45*+	4.68*+	9.25*+
Trichoderma	5.40*+	13.60*+	4.93*+	3.98*+

[a]Genera with >100 isolations from shells harvested at two locations; *Cylindrocladium*, an important pathogen of peanut, was included despite <100 isolations. ‡ = Significant interactions between harvest source and treatment according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).

[b]Mean percent isolation values (1,280 half shells) from four locations on two types of media. * = Significant differences across columns between two shell sources; + = significant differences between treatments according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).

Table 3. Effects of pod shell source and flutolanil treatment on isolation frequencies of *Fusarium* and *Rhizoctonia* spp. from peanut shells

	Detached pods		Harvested pods	
Taxa	Treated	Untreated	Treated	Untreated
Fusarium equiseti	18.90+[a]	12.60+	18.60+	14.60+
F. moniliforme	1.80	2.00	1.20	2.70
F. oxysporum	11.10*	13.00*	7.60*	7.40*
F. sambucinum	3.70	2.20	2.40	2.00
F. solani	17.60*	14.20*	13.80*	10.40*
Rhizoctonia oryzae	0.70	0.20	0.10	0.20
Rhizoctonia spp.				
AG-2-1	0.00	0.50	0.20	0.10
AG-2-2	0.60*	1.90*	0.40*	0.40*
AG-4	8.10*+	11.30*+	3.10*+	7.60*+
CAG-3	1.00	0.70	0.60	0.30
CAG-5	0.80	1.00	0.40	0.70

[a]Mean percent isolation values (1,280 half shells) from four locations on two types of media. * = Significant differences across columns between two shell sources; + = significant differences between treatments according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).

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