Survival of *Rhizoctonia solani* AG-4 in Residual Peanut Shells in Soil

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ABSTRACT

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The survival of *Rhizoctonia solani* AG-4 in residual peanut (*Arachis hypogaea*) shells was studied for 2 yr. Surface sterilized mature peanut pods colonized by *R. solani* AG-4 and untreated pods were placed separately in fiberglass-mesh bags and buried at 0, 7.6, and 25.4 cm depths in Tifton loamy sand in field microplots. Shells of pods were sampled at 6-mo intervals and assayed on tannic acid-benomyl agar for the recovery of *R. solani* AG-4 and other soilborne fungi. Recovery of *R. solani* AG-4 after 6 mo was greater ($P \leq 0.05$) from shells of treated pods placed on the soil surface than on those buried in the soil. Survival of *R. solani* AG-4 from shells at 12 mo was not different ($P \leq 0.05$) from those at 6 mo. *R. solani* AG-4 was isolated from less than 1% of the total shells after 18 and 24 mo. The fungi most commonly associated with untreated shells were species of *Fusarium, Gliocladium, Pythium, Trichoderma,* and binucleate *Rhizoctonia* (CAG-3 and CAG-5). *Fusarium* spp. were recovered at a higher ($P \leq 0.05$) level than other fungi during the last two sampling dates. *Fusarium* was the most abundant genus recovered at the conclusion of the study. Throughout the investigation, *Trichoderma* spp. were commonly isolated at a greater ($P \leq 0.05$) frequency from treated than from untreated shells and at increased burial depth. The increase of these fungi after 12 mo indicated that the inoculum potential of *R. solani* AG-4 remaining in the shells was diminishing. Burial of peanut pods 20–25 cm deep with a moldboard plow could effectively reduce the inoculum from the root zone of the following crop.

Residual peanut (*Arachis hypogaea* L.) pods are routinely left in the soil after harvest; the amount depends on the extent of physical damage, disease to the peg-pod connecting tissue, and the efficiency of harvesting equipment (13). In the cultivar Florunner, 630–778 kg/ha of pods were left in the soil after harvest (15). Peanut shells colonized by *Rhizoctonia solani* Kühn AG-4 and other soilborne pathogens can provide a large reservoir of inoculum (1,2,11). In a greenhouse study, peanut pods left in soil for 22 wk after harvest contained *R. solani* AG-4 which infected and killed soybean and peanut seedlings (3). Peanut shells can remain partially intact in the soil for approximately 2 yr after harvest, but most ungerminated seeds in buried pods are completely disintegrated after 1 yr. The rate of survival of *R. solani* AG-4 in peanut shells has not been reported.

Peanut shells contain pentosans (19.1%), starch (0.8%), sugar (2.3%), lignin (28.2%), fatty acids (1.2%), and cellulose (44.3%) (5). *R. solani* AG-4 can survive saprophytically in soil by utilizing cellulose from plant debris as a carbon source (6,8,9). Chung et al (4) showed that a hardwood-bark medium containing 45% cellulose (w/w) caused greater levels of disease when infested with fungi having high cellulase activity than did bark-compost medium with 5% cellulose. When increased levels of cellulose were added to the bark-compost medium, proportionally higher cellulase activity and disease occurred. Data from this study indicated that peanut shells may be a good substrate for the growth and survival of *R. solani* AG-4.

Soil depth may affect the survival of fungi in peanut shells. When peanut pods containing *R. solani* AG-4 were buried 5–10 cm deep, disease incidence on a subsequent crop of soybean was more severe than when pods were buried 0–5 or 10–15 cm deep (3). Burial of crop residues, such as peanut shells, by shallow disking 8–13 cm deep and turning 20–30 cm deep with a moldboard plow may affect disease incidence.

The objectives of this study were to determine the survival of *R. solani* AG-4 in peanut shells buried in soil at different depths and to document the occurrence of other potential pathogens and saprophytic fungi associated with *R. solani* AG-4 in decomposing peanut shells.

MATERIALS AND METHODS

On 2 November 1989, field microplots, which consisted of 91.4-cm-diameter open-ended fiberglass cylinders (surface area 0.7 m^2) placed 15.2 cm above and below the soil surface, were established at the Blackshank Farm, Coastal Plain Experiment Station, Tifton, Georgia. A split-split-plot experiment with a complete randomized-block design and four replications was established. Treatments represented whole plots, dates of sampling were subplots, and burial depths were sub-sub-plots. Twenty-six of the 32 microplots used were previously planted to peanut for 7 yr; six were planted to peanut for 6 yr and were fallow in 1989. The soil, Tifton loamy sand (fine loamy, siliceous, thermic, Plinthic Paleudults; pH 6.1, organic material 0.5% w/w), in each microplot was treated 4 wk prior to beginning the study with metam-sodium at 356.2 kg a.i./115.1 L water per hectare. This fumigant reduced population densities of fungi and bacteria up to 95% to a depth of 30 cm below the soil surface (D. K. Bell, *personal observation*). To obtain a uniform distribution of fungi in the microplots, 7.6 cm of topsoil was removed from each of the 26 continuous peanut microplots and mixed, and a 7.6-cm layer from the mixture was added to each of the six microplots left fallow in 1989. The excess soil was replaced in the 26 microplots (5.9-cm layer each) and thoroughly mixed into the upper 10–14 cm soil layer.

For the purpose of this paper, the fruit or pod is defined as containing both shells and seeds. A total of 6,000 peanut pods of cultivar Florunner was obtained from a field on the Coastal Plain Experiment Station that had been cropped to peanut for three continuous years. One-half of the pods (treated) were placed into four 10-L vacuum desiccators containing 50 ml of propylene oxide + 50 ml of distilled water in a dish, sealed, and placed under vacuum (71.7 kPa of mercury) at 22–23 C for 72 hr. To determine if sterilization was successful, seeds from 50 treated and 50 untreated pods were removed by hand, and subsamples of 50 treated and 50 untreated half shells were plated on modified tannic acid-benomyl agar medium (TABA) (15). Mycelium from resulting colonies was transferred to potato-dextrose agar (PDA) for further identification (1,2). The remaining treated pods were placed into 2.8-L sterilized Fernbach flasks for 48 hr and inoculated (five colonized PDA disks per flask) with an isolate of *R. solani* AG-4 obtained from a pod (RHS-109) grown locally and incubated for 2 wk. To determine if *R. solani* AG-4 colonized the shells of the pods, 50 half shells were soaked for 3 min in 0.53% aqueous sodium hypochlorite and plated onto TABA. Colonized pods were removed from the flasks, separated into groups of 50, and placed into 30 cm^2

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Table 1. Percentage of half shells yielding various fungi prior to initiation of *R. solani* AG-4 survival study

Taxa	Isolations (%)[z]
Alternaria spp.	38
Aspergillus flavus	1
Lasiodiplodia theobromae	5
Cirrenalia sp.	6
Cladosporium herbarum	1
Curvularia lunata	2
Epicoccum nigrum	1
Fusarium equiseti	7
Fusarium oxysporum	17
Geotrichum sp.	1
Mucor sp.	3
Neocosmospora vasinfecta	3
Nigrospora sphaerica	10
Oidiodendron tenuissimum	4
Phyllosticta sp.	1
Rhizoctonia spp.	
AG-4	3
AG 2-2	3
CAG-3	8
Rhizopus arrhizus	1
Sclerotium rolfsii	1
Tricoderma spp.	4
Waitea circinata	1
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[z] Percent isolations of fungi from 50 Florunner peanut untreated half shells grown on the tannic acid-benomyl agar medium.

polypropylene (mesh size 1 mm^2) bags and sealed with 15 cm of 18-gauge aluminum wire.

Untreated pods were stored for 24 hr at 2 C until grouped into lots of 50 pods each and placed into the mesh bags. A total of 96 bags (4,800 pods) was used in this study. Prior to burial of the mesh bags, hardware cloth (mesh size 5.0 mm^2) constructed cages were partially buried within each microplot at a depth of 30.0 cm. Both treated (48 bags) and untreated (48 bags) pods were placed within the cages, with three bags per cage at three soil depths: 0, 7.6, and 25.4 cm (3 bags in each microplot replicated four times). The top of each cage was then closed to prevent entry and pod damage by rodents throughout the study's duration. Five Florunner plants, which received 560 kg/ha 5-10-15 (N-P_2O_5-K_2O fertilizer + minor nutrients), were established in each microplot during early May 1990 and 1991. Recommended practices were followed for pest management in peanut production (12). The plants were lifted 145 days after planting and discarded.

Laboratory analysis. Every 6 mo after burial for 2 yr, four replicates per treatment were sampled for survival of *R. solani* AG-4. Twelve bags of pods from each treatment (four replicates from each soil depth) were lifted and brought to the laboratory for analysis. All pods were washed thoroughly in running tap water to remove excess soil and plant material. All pods were shelled and surface sterilized by the methods previously described. After 6 mo of burial the shells became progressively more decomposed, and the surface disinfestation time was reduced from 3.0 min to 30 sec for the remaining three sampling dates. The half shells or remaining fragments were incubated on TABA for 5 days, and colonies of fungi were subcultured onto PDA for identification. After 24 mo, the shells were extensively decayed, and fragments within the bags were less than 2.5 cm^2. These shell fragments were incubated on TABA as described for the first sampling date.

Statistical analysis. Analysis of variance (ANOVA) was conducted to compare the means of colonization treatment, sampling date, and soil depth, and their interactions (14). Means for treatment and soil-depth data were separated with the LSD test ($P \leq 0.05$). For the effects of sampling date on isolations, the Waller-Duncan k-ratio t test ($P \leq 0.05$) was used to separate means. Only sig-

Table 2. Survival of *Rhizoctonia solani* AG-4 and isolation of other fungi from peanut shells buried in soil for 2 yr

Depth (cm)	Taxa	Sampling time (mo)									
		Shells from untreated pods[w]					Shells from treated pods				
		0[x]	6	12	18	24	0	6	12	18	24
0	*Fusarium* spp.	0	0[y]	0	5.0	9.0	0	0	0	7.0	8.0
	Gliocladium spp.	24	0	0	0	<1	0	0	0	0	<1
	Pythium spp.	0	0	0	<1	0	0	0	0	1.0	0
	Rhizoctonia solani										
	AG 2-2	3	0	<1	0	0	0	0	<1	0	0
	AG-4	3	1.0	<1	1	<1	98	7.3	3.7	1.0	<1
	Binucleate *Rhizoctonia* spp.										
	CAG-3	8	<1	0	<1	<1	0	0	0	0	0
	CAG-5	0	0	0	<1	0	0	0	0	<1	0
	Trichoderma spp.	4	<1	1.0	4.0	<1	0	1.2	5.0	3.0	6.0
7.6	*Fusarium* sp.		0	<1	1.0	8.0		0	<1	7.0	5.0
	Gliocladium spp.		0	0	0	<1		0	0	0	1.0
	Pythium spp.		0	0	2.0	0		0	0	<1	0
	Rhizoctonia solani										
	AG 2-2		0	0	0	0		0	<1	0	0
	AG-4		<1	0	<1	0		2.0	4.0	<1	<1
	Binucleate *Rhizoctonia* spp.										
	CAG-3		0	0	<1	0		0	0	<1	0
	CAG-5		0	0	0	0		0	0	0	0
	Trichoderma spp.		<1*[z]	1.0*	3.0*	3.0*		5.0*	2.1*	5.0*	5.0*
25.4	*Fusarium* spp.		0	<1	7.0	6.0		0	<1	8.0	5.0
	Gliocladium spp.		0	0	0	<1		0	0	0	<1
	Pythium spp.		0	0	<1	0		0	0	2.0	0
	Rhizoctonia solani										
	AG 2-2		<1	0	0	0		0	0	0	0
	AG-4		0	<1	3.0	0		0	<1	3.0	<1
	Binucleate *Rhizoctonia* spp.										
	CAG-3		0	0	0	0		0	0	0	0
	CAG-5		0	0	<1	0		0	0	0	0
	Trichoderma spp.		0	<1	2.3	2.4		6.8	2.2	<1	1.3

[w] Untreated pods = naturally infested with *R. solani* AG-4 and other fungi. Treated pods = artificially infested with *R. solani* AG-4.

[x] All data in the 0 column are based upon a preliminary sampling (50 pods) of untreated and treated pods and were not included in the statistical analysis.

[y] Percentage of peanut half shells (300 per sampling date) yielding cultures.

[z] Percentage of the fungi isolated was significantly different between treatments. Means by a corresponding time were separated by LSD ($P \leq 0.05$).

Table 3. Effect of sampling time on the percentage of different fungal genera isolated from residual peanut shells

Sampling time (mo)	Fungal genera[x]				
	Fusarium	*Pythium*	BR[y] CAG-3	BR[y] CAG-5	*Gliocladium*
6	0.00 b[z]	0.00 b	0.00 b	0.00 b	0.00 b
12	0.50 b	0.00 b	0.00 b	0.00 b	0.00 b
18	20.79 a	2.92 a	0.42 a	0.21 a	0.00 b
24	20.67 a	0.00 b	0.00 b	0.00 b	1.75 a

[x] Only those genera that changed significantly over time are shown.
[y] BR = Binucleate *Rhizoctonia* spp.
[z] Means within columns followed by different letters are signficantly different ($P \leq 0.05$) according to Waller-Duncan k-ratio t test.

nificant ($P \leq 0.05$) differences will be discussed unless stated otherwise.

RESULTS AND DISCUSSION

No fungi or bacteria were recovered after the sterilization of pods with propylene oxide prior to colonization by *R. solani* AG-4. After the shells were artificially inoculated with *R. solani,* the fungus was recovered from 96% of the shells. Thus, the colonized shells contained sufficient inoculum to determine the ability of the pathogen to survive in soil for 2 yr.

In a preliminary test, over 20 species of fungi were isolated from 50 untreated half shells (Table 1). The common fungi observed were *Alternaria, Fusarium,* and *Rhizoctonia* spp. anastomosis groups AG-4, AG 2-2, and CAG-3.

For the duration of the study, isolations of *R. solani* AG-4 in shells from treated pods at 0 depth decreased over time, but those in untreated shells did not (Table 2). In general, isolations of *Fusarium* spp. from shells of treated and untreated pods increased at 0 and 7.6 cm with time.

Isolations of *Trichoderma* spp. varied with time, treatment, and depth of burial, but no consistent trend was noted (Table 2). *Trichoderma* spp. were more common in treated than in untreated shells. *Trichoderma,* which can decompose cellulose, became established more readily in pods colonized with only *R. solani* AG-4 (12). Rapid growth by *Trichoderma* and other potentially antagonistic fungi such as *Gliocladium* could be significant deterrents to disease if pods were colonized early.

The recovery of species of *Fusarium, Pythium, Gliocladium,* and binucleate *Rhizoctonia* CAG-3 and CAG-5 was influenced by the sampling date (Table 3). *Fusarium* and *Gliocladium* spp. were isolated more frequently than other fungi at the end of 2 yr. Isolation of *Pythium* spp. and of binucleate *Rhizoctonia* CAG-3 and CAG-5 was higher after 18 mo than for other sampling dates. The recovery of *Fusarium* spp. also was higher at 18 and 24 mo than at 6 and 12 mo. No differences by time were observed in the isolation of *R. solani* AG-4 from untreated pods. Species of *Fusarium* and *Gliocladium* may have been secondary colonizers of shells after the primary cellulose degrading fungi, *R. solani* AG-4 and *Trichoderma* spp., had partially depleted the food source.

At the 6-mo sampling, *R. solani* AG-4 was isolated more frequently from treated shells nearest the surface (12.6%), compared to 3.0% for the 7.6-cm and 3.8% for the 25.4-cm soil depths. Means for the remaining depths and sample periods were not different. Over the 2-yr period, there was a trend toward decreasing isolations of *R. solani* AG-4 at all depths, with a notable exception for 18 mo at 25.4 cm, where isolations of the fungus sharply increased.

These results indicated that *R. solani* AG-4 could survive in peanut shells up to, and probably longer than, 2 yr. However, after the first year, inoculum potential was much lower than after 6 mo. Depletion of part of the cellulose food base may be critical to the survival of *R. solani* AG-4 (1,4,6,10). It is reasonable to hypothesize that the presence of antagonistic organisms as well as the reduced food reserve rapidly reduced the population. At the lower depth, *R. solani* AG-4 also could be suppressed by higher CO_2 concentrations (7). Populations decline with depth in cultivated soil primarily because of the high CO_2 levels (16). At greater depths, nutrient depletion may have occurred at a slower rate, and *R. solani* AG-4 may have survived longer in shells but at a reduced population density.

Results from this study show that peanut producers should utilize appropriate management practices in fields previously known to have high levels of *R. solani* AG-4. Peanut shells left in the soil after harvest can normally be found in the actively growing root zone (17). Management strategies to remove habitats of *R. solani* AG-4 include the use of a moldboard plow, which inverts soil up to 30 cm deep and removes the shell debris and pathogen from the root zone. Debris such as soybean and peanut stubble or pods used in conservation tillage practices can harbor *R. solani* AG-4. Concentrations of the pathogens occur in the 5–15 cm soil layer; but a crop rotation to nonhosts such as wheat, rye, and other small grains was reported to inhibit *R. solani* AG-4 on debris near the soil surface (17).

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