Interactions of Pythium myriotylum with Several Fungi in Peanut Pod Rot

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ABSTRACT

Interactions of *Pythium myriotylum* with several fungi in pod rot of peanut were evaluated by exposing pods to fungal pathogens alone or in various combinations in vitro or at defined inoculum densities in soil. The exposure of attached or detached pods to *Rhizoctonia solani* prevented or reduced the development of rot in pods later exposed to *P. myriotylum* in soil or in vitro. High populations of *Macrophomina phaseolina* nullified the antagonistic effect of *R. solani* on *P. myriotylum* if the pods were grown in soil, but not if the pods were grown in vermiculite. The isolation frequency of *P. myriotylum* from pods was reduced after exposure to soil containing *R. solani*. This effect was most striking with attached or detached pods which were exposed successively to *R. solani* in soil or in vitro prior to exposure to *P. myriotylum* in soil. *Rhizoctonia solani*, on the other hand, was isolated much less frequently or could not be isolated at all from attached or detached pods exposed to *P. myriotylum* prior to exposure to *R. solani*. If pods were exposed to *Fusarium solani* or *Trichoderma viride* in vitro prior to *R. solani* and then *P. myriotylum* in soil, pod rot occurred but no *P. myriotylum* and little or no *R. solani* were isolated from the pods. However, when pods were exposed to *R. solani* in vitro prior to *F. solani* or *T. viride* and then *P. myriotylum* in soil, pod rot was not greater than in the control and *R. solani* but not *P. myriotylum* was isolated from the pods.

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Additional key words: Fusarium solani, Macrophomina phaseolina, Rhizoctonia solani, Trichoderma viride.

Many plant pathogenic fungi are associated with pod rot of peanut (*Arachis hypogaea* L.) and about 110 genera and 200 species of fungi have been isolated from peanut pods (4, 9, 10). Interactions among some of the isolated fungi have been reported (5, 6, 11, 17). *Trichoderma viride* Pers. ex Fries reduced colonization of immature and mature pericarps by *Aspergillus flavus* (Link.) Fr., but this antagonism was nullified in the presence of *Penicillium funiculosum* Thom which also stimulated colonization of mature pericarps and testae by *A. flavus* (17). *Aspergillus flavus* reduced the growth and spread of *Macrophomina phaseolina* (Tassi) Goid when pods and kernels were inoculated simultaneously with both fungi (11). Garren (6) considered *Pythium myriotylum* Drechs. and *Rhizoctonia solani* Kuhn capable of causing peanut pod breakdown (pod rot), and suggested that competition between these fungi resulted in domination by *P. myriotylum* in causing pod rot under most conditions. In earlier work, greater competitive ability of *T. viride* over *R. solani* in decaying pods made it difficult to assess the role of *R. solani* in the disease (5). Frank (2) found that *P. myriotylum* and *Fusarium solani* (Mart.) App. & Wr. emend Snyd. & Hans. interact synergistically in peanut pod rot, and concluded that *F. solani* not only predisposed pods to attack by *P. myriotylum* but was also involved in the final disintegration of diseased pods.

Pythium myriotylum, R. solani, F. solani, M. phaseolina, and *T. viride* were consistently isolated from sound and rotted peanut pods from a farm with a high incidence of pod rot in Levy County, Florida (3). The objective of this study was to evaluate the interactions of these fungi at defined or mass inoculum densities in pod rot of peanut.

MATERIALS AND METHODS.—*Inoculum production.*—The fungi used throughout this investigation were isolated from rotted peanut pods. Hyphal-tipped cultures of *Fusarium solani, Macrophomina phaseolina, Pythium myriotylum, Rhizoctonia solani,* and *Trichoderma viride* were maintained on V-8 juice agar (200 ml Campbell's V-8 juice, 4.5 g $CaCO_3$, 17 g Difco agar, and 800 ml distilled water).

Fungal inoculum for soil infestation was prepared by adding a 4-mm diameter disk cut from the margin of a 48-hour-old V-8 juice agar culture of each fungus to a 250-ml Erlenmeyer flask containing 50 ml of V-8 juice broth (100 ml Campbell's V-8 juice, 4.5 $CaCO_3$, and 800 ml distilled water). The flasks were maintained at approximately 25 C under continuous light (1750 lx at the level of the cultures) for 15 days.

Inoculum densities in soil.—Inoculum densities of the fungal pathogens in soil were prepared by adding specific numbers of spores or resting structures to soil. Arredondo fine sand with a pH of 6.5 (measurement obtained from a 1:2 suspension of soil in 0.01 M $CaCl_2$) was used throughout this study after it was autoclaved twice for 4 hours at 24-hour intervals.

Oospores of *P. myriotylum* were obtained by washing five mats of the fungus from V-8 juice broth cultures with sterile distilled water and blending the cultures in 50 ml of sterile distilled water for 20 seconds at maximum intensity in a micro-blender. The resulting suspension was subjected to 50% maximum sonification with a Biosonik III ultrasonic system for 40 seconds to leave only oospores as viable propagules in the suspension. The number of oospores in the suspension was determined by counting six fields for each of 12 samples in a haemocytometer.

Mycelial mats of *F. solani* and *T. viride* were blended in their own culture media for 45 seconds at maximum intensity in a micro-blender. The suspension was passed

through a 45-μm sieve and centrifuged at 1,000 *g* for 5 minutes. The pellet was resuspended in sterile distilled water and the number of conidia per ml was estimated by counting six fields for each of 12 samples in a haemocytometer.

Mycelial mats containing sclerotia of *R. solani* and *M. phaseolina* were blended in sterile tap water for 45 seconds at maximum intensity in a blender, and the resulting suspensions were passed through nested 125- and 260-μm sieves. The sclerotia were retained and the mycelia fragments were removed by exposure to a high pressure water spray. After resuspending the sclerotia in tap water, the number of sclerotia/ml was estimated by counting the sclerotia in twelve 210 mm^2 microscope fields per sample.

Peanut pod production.—Florunner peanut plants were grown in the greenhouse at 27 to 35 C. Peanut seeds were soaked in running tap water for 24 hours, surface disinfested with 1.0% sodium hypochlorite for 40 seconds, rinsed three times with sterile tap water, and placed to germinate in moist, sterile vermiculite in petri dishes. One germinated seed was placed in the top of an open-ended tube (25 cm long and 5 cm in diameter) filled with soil. Each tube was placed on top of soil contained in a 20-liter can filled to 0.75 of its capacity. This elevated system allowed the roots to grow down through the tube into the soil, and maintained the foliage high enough to provide space for chambers which contained the peanut fruit. Twenty-five g of a 15:30:15 (N-P-K) fertilizer formulation was applied to each plant 15 days after planting. The plants were inoculated with *Rhizobium leguminosarum* Frank 21 days after planting, and 9 g of gypsum was added to the soil under each plant at flowering (about 4 weeks after planting). All plants were sprayed to run off periodically with Kelthane (Kelthane EC, 18.5%, 1.5ml/liter) to control mites and monthly with triphenyl tin hydroxide (2.5 g/liter) to reduce the incidence of Cercospora leaf spot.

For studies with pods attached to plants, each peg (stalk-like young ovary) was surface disinfested with 1.0% sodium hypochlorite and rinsed in sterile tap water. The pegs were then introduced into 50-ml test tubes that were covered with parafilm and aluminum foil and contained either autoclaved vermiculite (10% moisture v/v and 0.1 g of gypsum per tube) or soil (10% moisture v/v and 0.1 g of gypsum per tube). Pegs disinfested in the same way were also induced to grow in 200-ml polystyrene cups containing autoclaved soil.

Interaction of P. myriotylum and R. solani on attached and detached peanut pods.—Pods grown in autoclaved soil in polystyrene cups for 6 weeks were carefully removed from the soil and six attached pods were introduced into each of seven cups containing noninfested soil for a control or into each of 28 cups with infested soil. Soil in 14 cups was directly infested with 200 oospores of *P. myriotylum*/g of soil, and soil in an additional 14 cups was directly infested with 10 sclerotia of *R. solani*/g of soil. After 20 days, pods were removed from seven cups with *P. myriotylum* infested soil and placed in cups containing soil infested with *R. solani*. One-half of the cups with pods exposed to *R. solani* for 20 days were transferred to cups containing *P. myriotylum*. All cups were maintained for an additional 20 days in the greenhouse.

For studies with detached pods, mature pods were removed from the autoclaved soil and five detached pods were placed in each of eight polystyrene cups for each treatment. The treatments consisted of a noninfested soil control, soil infested with 200 oospores of *P. myriotylum*/g of soil, and soil infested with 10 sclerotia of *R. solani*/g of soil. The cups were covered with aluminum foil and maintained at 30 C in a growth chamber. After 4 days, pods in eight cups containing *P. myriotylum* were transferred into soil infested with *R. solani*, and pods in one-half of the cups with *R. solani* were transferred to soil containing *P. myriotylum*. All cups were incubated at 30 C for an additional 4 days.

Interactions of Fusarium solani, Macrophomina phaseolina, Pythium myriotylum, and Rhizoctonia solani at high and low inoculum densities on detached peanut pods.—After 8 to 9 weeks of growth in autoclaved soil or vermiculite, six mature peanut pods were detached and placed in each of four cups for each treatment. The treatments consisted of a noninfested soil control and soil infested with factorial combinations of the following fungi at low and high inoculum densities: (i) *M. phaseolina* at 100 or 200 sclerotia/gram of soil; (ii) *R. solani* at 1 or 10 sclerotia/g of soil; and (iii) *F. solani* plus *P. myriotylum* at 1,000 conidia/g of soil plus 100 oospores/g of soil, respectively, or 10,000 conidia/g of soil plus 1,000 oospores/g of soil, respectively. *Pythium myriotylum* was combined with *F. solani* in these experiments because these fungi interact synergistically (2, 3) and, thus, should induce a high incidence of pod rot. The cups were incubated at 30 C for 12 days.

Successive in vitro inoculations with detached pods.—Mature pods from autoclaved soil were surface disinfested with 1.0% sodium hypochlorite for 40 seconds and rinsed three times in sterile distilled water. Four pods were placed on each of 12 V-8 juice agar cultures (48-hour-old) of one of the following fungi: *F. solani, M. phaseolina, P. myriotylum,* or *R. solani*. After 36 hours at 30 C in the dark, 12 pods were transferred from cultures of one fungus to 48-hour-old cultures of each of the other three fungi for an additional 72 hours. The remaining 12 pods exposed to each fungus were transferred to fresh 48-hour-old cultures of the same fungus with which they were originally inoculated.

Successive exposure of detached pods to fungal cultures and infested soil.—After surface disinfestation with 1.0% sodium hypochlorite for 40 seconds and three rinses in sterile distilled water, 100 pods were placed in 25 petri plates containing 48-hour-old V-8 juice agar cultures of one of the following fungi: *F. solani, P. myriotylum, R. solani* or *T. viride*. After 72 hours at 30 C in the dark, 25 pods from cultures of each fungus were placed in five cups containing soil infested with one of the other fungi. The infested soil contained 1,000 conidia of *F. solani*/g of soil, 200 oospores of *P. myriotylum*/g of soil, 10 sclerotia of *R. solani*/g of soil, or 30,000 conidia of *T. viride*/g of soil. The cups were covered with aluminum foil and maintained at 30 C for 4 days. The pods were then transferred to cups containing 200 oospores of *P. myriotylum*/g of soil and incubated at 30 C for an additional 4 days.

Disease evaluation.—The evaluation of disease severity was based either on the percentage of pod rot or on an index ranging from 1 for healthy pods to 5 for

completely blackened pods. Frequency of isolation of pathogenic fungi from pods was evaluated following surface disinfesting pods with 1.0% sodium hypochlorite for 40 seconds, rinsing three times with sterile distilled water, and plating on the following selective media: The pimaricin-vancomycin-PCNB medium of Tsao and Ocana (15) for *P. myriotylum,* a selective medium for *R. solani* (12), and potato-dextrose agar, acidified with lactic acid to pH 4.0 for the isolation of *M. phaseolina, F. solani* and *T. viride.*

RESULTS.—*Interactions of P. myriotylum and R. solani with attached and detached peanut pods.*—In both attached and detached fruits, pod rot was less severe when pods were exposed to *R. solani* prior to *P. myriotylum* than when pods were exposed to *P. myriotylum* alone (Table 1). The antagonistic effect of *R. solani* on *P. myriotylum* was also observed in attached pods exposed to *P. myriotylum* before *R. solani,* but not with detached pods.

Symptoms of pod rot were not evident on detached pods from soil infested only with *R. solani,* but were significantly greater on attached pods with *R. solani* than in the control.

Pythium myriotylum was isolated only from attached pods recovered from soil infested with *P. myriotylum* alone. With detached pods, *P. myriotylum* was isolated from 90 to 100% of the pods initially exposed to soil infested with *P. myriotylum* and from 50% of the pods initially exposed to *R. solani.* Over 50% of the attached or detached pods initially exposed to *R. solani* yielded the fungus, but *R. solani* was not isolated from pods exposed to *P. myriotylum* prior to *R. solani.*

Interactions of F. solani, M. phaseolina, P. myriotylum and R. solani at high and low inoculum densities with detached peanut pods.—When pods grown in autoclaved soil were detached and placed in infested soil, the incidence of pod rot with high inoculum levels of *P. myriotylum* plus *F. solani* was reduced significantly in the presence of *R. solani* when low levels of *M. phaseolina* were also present, but not when high levels of *M. phaseolina* were present (Table 2). A high percentage of pod rot also occurred with high levels of *P. myriotylum* plus *F. solani,* and with low levels of *P. myriotylum* plus *F. solani* in combination with a high level of *M. phaseolina* and a low level of *R. solani.* An antagonistic effect was observed with all other combinations involving low levels of *P. myriotylum* plus *F. solani.* The percentages of pod rot with high levels of *R. solani* and *M. phaseolina* in the absence of *P. myriotylum* plus *F. solani* was not significantly greater than the control.

Pythium myriotylum was isolated only from detached pods from soil containing *P. myriotylum* plus *F. solani* in the absence of both *R. solani* and *M. phaseolina.* The isolation of *R. solani* was not correlated with any specific combinations of high or low inoculum densities of the fungi tested. The percentage of isolation of *M. phaseolina* was consistently greater from pods in soil with high levels of sclerotia than from pods in soil with low levels of sclerotia. Pods from soil containing *R. solani* and *M. phaseolina* yielded significantly fewer colonies of *F. solani* than pods from soil containing *P. myriotylum* plus *F. solani* without the other fungi.

When pods were grown in vermiculite prior to exposure to infested soil, significant pod rot occurred only in soil containing *P. myriotylum* and *F. solani* in the absence of *R. solani* and *M. phaseolina* (Table 3). *Pythium myriotylum* was recovered only from pods exposed to *P. myriotylum* and *F. solani* alone; in contrast, *R. solani* was isolated from all other treatments except the control.

Successive in vitro inoculations with detached peanut pods.—The inoculation of peanut pods with *P. myriotylum* alone or the successive in vitro inoculation with *P. myriotylum* and other fungi resulted in

TABLE 1. Pod rot severity and frequency of fungi isolated from attached and detached peanut pods after exposure to soil infested with known quantities of reproductive structures of *Pythium myriotylum* or *Rhizoctonia solani*[w]

Treatments[x]		Pod rot index[y]	Frequency of isolation (%)	
Initial exposure	Second exposure		*P. myriotylum*	*R. solani*
Attached Pods				
Noninoculated	Noninoculated	1.0 a[z]	...	...
R. solani	*R. solani*	1.5 b	...	62.5
R. solani	*P. myriotylum*	1.6 b	0.0	52.5
P. myriotylum	*R. solani*	1.8 b	0.0	0.0
P. myriotylum	*P. myriotylum*	3.6 c	40.0	...
Detached Pods				
Noninoculated	Noninoculated	1.0 a[z]	0.0	0.0
R. solani	*R. solani*	1.0 a	0.0	70.0
R. solani	*P. myriotylum*	1.8 a	50.0	80.0
P. myriotylum	*R. solani*	3.6 b	100.0	0.0
P. myriotylum	*P. myriotylum*	4.0 b	90.0	0.0

[w]Soil infested with *P. myriotylum* at 200 oospores/g of soil, and *R. solani* at 10 sclerotia/g of soil.

[x]Attached pods were grown for 6 weeks in autoclaved soil and were then exposed to soil containing one fungus for 20 days and finally were shifted to soil containing the same or another fungus for 20 days. Detached pods were initially grown in autoclaved soil for 9 weeks and were then detached and exposed to soil containing one fungus for 4 days and finally were shifted to soil containing the same or another fungus for 4 days.

[y]Pod rot index: 1 = healthy pod; 5 = completely blackened pod.

[z]Values followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

TABLE 2. Incidence of pod rot and isolation frequency of fungi from detached mature peanut pods after 8 weeks growth in autoclaved soil and 12 days exposure to combinations of fungi at high and low inoculum levels in soil infested with known quantities of fungal reproductive structures[y]

Inoculum level				Frequency of isolation (%)			
Rhizoctonia solani	*Macrophomina phaseolina*	*Pythium myriotylum* + *Fusarium solani*	Pod rot (%)	*P. myriotylum*	*R. solani*	*M. phaseolina*	*F. solani*
High	High	High	23.8 b[z]	0.0 a	33.3 b	18.7 b	38.0 b
Low	High	High	31.2 b	0.0 a	18.7 b	12.5 b	31.2 b
High	Low	High	12.5 a	0.0 a	6.2 a	4.5 a	37.5 b
Low	Low	High	12.5 a	0.0 a	0.0 a	6.2 a	56.2 b
High	High	Low	6.2 a	0.0 a	0.0 a	25.0 b	31.2 b
Low	High	Low	25.0 b	0.0 a	25.0 b	25.0 b	37.5 b
High	Low	Low	18.7 a	0.0 a	0.0 a	4.7 a	62.5 b
Low	Low	Low	18.7 a	0.0 a	12.5 b	0.0 a	52.2 b
High	High	None	12.5 a	0.0 a	12.5 b	18.7 b	0.0 a
None	None	High	37.5 b	31.2 b	0.0 a	0.0 a	87.5 c
None	None	Low	31.2 b	25.0 b	0.0 a	0.0 a	93.7 c
None	None	None	0.0 a	0.0 a	0.0 a	0.0 a	12.5 a

[y]Inoculum levels were: *R. solani,* high = 10 sclerotia/g of soil (sgs), and low = 1 sgs; *M. phaseolina,* high = 200 sgs, and low = 100 sgs; *P. myriotylum* plus *F. solani,* high = 1,000 oospores plus 10,000 conidia/g of soil, respectively, and low = 100 oospores plus 1,000 conidia/g of soil, respectively.

[z]Any pair of values in a column followed by the same letter are not significantly different ($P = 0.05$) when compared by the contingency chi-square test.

TABLE 3. Incidence of pod rot and isolation frequency of fungi from detached peanut pods after 8 weeks growth in autoclaved vermiculite and 12 days exposure to combinations of fungi at high and low inoculum levels in soil infested with known quantities of the fungal reproductive structures[y]

Inoculum level			Pod rot (%)	Frequency of isolation (%)	
Rhizoctonia solani	*Macrophomina phaseolina*	*Pythium myriotylum* + *Fusarium solani*		*P. myriotylum*	*R. solani*
High	High	High	11.2 a[z]	0.0 a	66.6 b
Low	High	High	22.2 a	0.0 a	100.0 b
High	Low	High	11.1 a	0.0 a	100.0 b
Low	Low	High	11.1 a	0.0 a	100.0 b
High	High	Low	0.0 a	0.0 a	77.7 b
Low	High	Low	0.0 a	0.0 a	100.0 b
High	Low	Low	0.0 a	0.0 a	77.7 b
Low	Low	Low	0.0 a	0.0 a	88.8 b
High	High	None	0.0 a	0.0 a	77.7 b
None	None	High	100.0 b	100.0 a	0.0 a
None	None	Low	77.7 b	44.4 a	0.0 a
None	None	None	0.0 a	0.0 a	0.0 a

[y]Inoculum levels were: *R. solani,* high = 10 sclerotia/g of soil (sgs), and low = 1 sgs; *M. phaseolina,* high = 200 sgs, and low = 100 sgs; *P. myriotylum* plus *F. solani,* high = 1,000 oospores plus 10,000 conidia/g of soil, respectively, and low = 100 oospores plus 1,000 conidia/g of soil, respectively.

[z]Any given pair of values in a column followed by the same letter are not significantly different ($P = 0.05$) when compared by the contingency chi-square test.

significantly higher severity of pod rot than in noninoculated controls (Table 4). When pods were inoculated with *R. solani* prior to inoculation with *P. myriotylum,* however, the severity of pod rot was not significantly different from the control.

Inoculation of pods with *P. myriotylum* before or after exposure of pods to other fungi resulted in a high isolation frequency of *P. myriotylum,* except when it was preceded by *R. solani.* The isolation frequency of *R. solani* was generally high, but was reduced when the pods were initially exposed to *P. myriotylum* or *F. solani. Fusarium solani* usually was isolated from 100% of the pods exposed to this fungus, but 33% of the control pods also yielded *F. solani. Fusarium solani* was not isolated from pods from the other treatments not exposed to this fungus.

Successive exposure of detached peanut pods to fungal cultures and infested soil.—Inoculation of pods in vitro with *F. solani, T. viride,* or *P. myriotylum* followed by exposure in soil to *F. solani, R. solani, T. viride,* or *P. myriotylum* and a final exposure period to *P. myriotylum* infested soil resulted in significantly higher severity of pod rot than in other treatments (Table 5). In vitro inoculations with *R. solani* followed by exposure in soil to *F. solani, P. myriotylum, R. solani,* or *T. viride,* and a final exposure to *P. myriotylum* infested soil resulted in a pod rot severity that was not significantly greater than that in the control. The exposure of pods to *R. solani* in

TABLE 4. Influence of successive 36-hour exposures of detached peanut pods to fungal cultures on pod rot severity and isolation frequency of fungi

Inoculation		Pod	Frequency of isolation (%)			
Initial exposure	Secondary exposure	rot index[y]	*Pythium myriotylum*	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Fusarium solani*
P. myriotylum	*F. solani*	5.0 a[z]	100.0	...	...	100.0
F. solani	*P. myriotylum*	4.3 ab	66.5	...	...	100.0
P. myriotylum	*M. phaseolina*	4.2 ab	78.5	...	0.0	0.0
P. myriotylum	*R. solani*	4.1 ab	90.0	5.0	...	0.0
P. myriotylum	*P. myriotylum*	4.1 ab	86.3	...	...	0.0
M. phaseolina	*P. myriotylum*	3.0 b	100.0	...	7.1	0.0
F. solani	*M. phaseolina*	2.3 c	...	...	0.0	100.0
F. solani	*R. solani*	2.3 c	...	23.6	...	100.0
R. solani	*F. solani*	2.1 c	...	45.5	...	78.5
F. solani	*F. solani*	2.1 c	...	...	...	100.0
R. solani	*P. myriotylum*	1.9 c	41.5	95.5	...	0.0
R. solani	*M. phaseolina*	1.8 c	...	78.5	52.2	0.0
M. phaseolina	*R. solani*	1.4 c	...	71.4	37.9	0.0
R. solani	*R. solani*	1.2 c	...	92.8	...	0.0
M. phaseolina	*F. solani*	1.2 c	...	...	16.5	100.0
M. phaseolina	*M. phaseolina*	1.0 c	...	...	83.0	0.0
None	None	1.0 c	0.0	0.0	0.0	33.3

[y]Pod rot index: 1 = healthy pod; 5 = completely blackened pod.
[z]Values followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 5. Effect of in vitro inoculation of detached peanut pods for 72 hours followed by two successive 4-day exposures of pods to soil infested with various fungal pathogens[x] on severity of pod rot and isolation frequency of fungi from mature pods initially grown in autoclaved soil

In vitro inoculation	First soil exposure	Second soil exposure	Pod rot index[y]	Frequency of isolation (%)			
				P. myriotylum	*R. solani*	*F. solani*	*T. viride*
F. solani	*T. viride*	*P. myriotylum*	4.64 a[z]	68.9	...	86.9	52.1
T. viride	*F. solani*	*P. myriotylum*	4.60 a	90.4	...	19.0	80.9
F. solani	*P. myriotylum*	*P. myriotylum*	3.27 a	77.2	...	81.8	0.0
F. solani	*F. solani*	*P. myriotylum*	4.15 a	90.9	...	81.8	0.0
F. solani	*R. solani*	*P. myriotylum*	4.14 a	0.0	4.7	100.0	14.2
T. viride	*P. myriotylum*	*P. myriotylum*	4.10 a	68.1	...	40.9	68.1
P. myriotylum	*T. viride*	*P. myriotylum*	4.08 a	100.0	...	0.0	59.0
None	*P. myriotylum*	*P. myriotylum*	3.96 a	100.0	...	8.0	12.0
P. myriotylum	*P. myriotylum*	*P. myriotylum*	3.96 a	100.0	...	8.0	12.0
P. myriotylum	*F. solani*	*P. myriotylum*	3.90 a	95.4	...	77.2	0.0
T. viride	*T. viride*	*P. myriotylum*	3.82 a	86.3	...	13.6	59.0
T. viride	*R. solani*	*P. myriotylum*	3.80 a	0.0	0.0	12.5	62.5
P. myriotylum	*R. solani*	*P. myriotylum*	3.64 a	60.8	0.0	34.7	17.3
R. solani	*F. solani*	*P. myriotylum*	2.09 b	0.0	45.4	68.1	9.0
R. solani	*P. myriotylum*	*P. myriotylum*	1.91 b	80.0	35.0	40.0	10.0
R. solani	*R. solani*	*P. myriotylum*	1.85 b	16.0	60.0	28.0	32.0
R. solani	*T. viride*	*P. myriotylum*	1.80 b	0.0	59.0	18.1	63.6
F. solani	*F. solani*	*F. solani*	2.16 b	...	...	100.0	0.0
R. solani	*R. solani*	*R. solani*	1.33 b	...	60.0	40.0	40.0
T. viride	*T. viride*	*T. viride*	1.23 b	...	...	...	100.0
None	None	None	1.23 b	...	...	16.0	16.0

[x]Inoculum levels were: *Pythium myriotylum*, 200 oospores/g of soil; *Rhizoctonia solani*, 10 sclerotia/g of soil; *Fusarium solani*, 1,000 conidia/g of soil; and *Trichoderma viride* 30,000 conidia/g of soil.
[y]Pod rot index: 1 = healthy; 5 = completely blackened pod.
[z]Values followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

soil prior to *P. myriotylum* exposure did not result in reduction of pod rot severity if the initial in vitro inoculation was with *F. solani*, *T. viride*, or *P. myriotylum*. None of the treatments with individual pathogens alone except inoculation with *P. myriotylum* resulted in significant levels of pod rot over that in the control.

The isolation frequency of *P. myriotylum* was over 50% in all treatments in which it was included, except in most sequences in which exposure to *P. myriotylum* was preceded by exposure to *R. solani*. However, the sequence *R. solani* - *P. myriotylum* - *P. myriotylum* yielded 80% isolation of *P. myriotylum*. No *P. myriotylum* was isolated after the sequences *F. solani* - *R.*

solani - *P. myriotylum* or *R. solani* - *T. viride* - *P. myriotylum*. In the sequence of inoculation with *R. solani* - *R. solani* - *P. myriotylum*, only 16% of the pods yielded *P. myriotylum*. *Rhizoctonia solani* was isolated from over 35% of pods in all treatments in which pods were inoculated with the fungus in vitro. No *R. solani* could be isolated from pods exposed to it in soil if they were first inoculated in vitro with *T. viride* or *P. myriotylum*. Only 4.7% of the pods inoculated with *F. solani* and exposed to *R. solani* and then *P. myriotylum* yielded *R. solani*. The isolation frequency of *F. solani* from pods exposed to this fungus was above 68% except when soil exposure was preceded by in vitro inoculation with *T. viride*. *Fusarium solani* was isolated from 0 to 41% of the pods in treatments in which it had not been included. The frequency of isolation of *T. viride* from pods in treatments that included exposure to it varied from 52 to 100%, and ranged from 0 to 32% from pods in treatments that had not included artificial infestation.

DISCUSSION.—Although *P. myriotylum* and *R. solani* are two of the most important pathogens involved in peanut pod rot, interactions between these two fungi have received little critical attention. Garren (6) observed antagonism between *R. solani* and *P. myriotylum* and found that indigenous populations of *P. myriotylum* dominated over introduced *R. solani* in causing pod rot. In fields where both pathogens are present, one fungus may be isolated more frequently from rotted pods one year, and the other fungus may predominate another year (3, 4, 16). The results of this study, as well as those of Garren (7), indicate that damage is much more severe with *P. myriotylum* than with *R. solani*. The isolate of *R. solani* used in this experiment caused a minimal but significant amount of rot in attached pods, but did not cause damage to detached pods in any of the experiments. Furthermore, *R. solani* acted as an antagonist to *P. myriotylum;* the colonization of attached or detached pods by *R. solani* prevented or reduced the development of pod rot in pods later exposed to *P. myriotylum* in vitro or in soil.

The severity of pod rot and the frequency of isolation of fungi from pods are influenced strongly by the sequence of exposure of pods to the various fungi, and by the earliest invaders of the pod. Although Garren (8) did not specifically indicate which fungi might be antagonistic to *P. myriotylum,* he did observe that *P. myriotylum* survived longer in soil in which it was not indigenous than in soil to which it was native, and he suggested that organisms antagonistic to *P. myriotylum* were present in the native soil. Garren (5) also recognized that antagonistic organisms colonizing pods could be part of the natural barrier to invasion by phytopathogenic fungi. Flowers and Littrell (1, 14) reported that one isolate of *R. solani* was antagonistic to *Pythium aphanidermatum* (Edson) Fitz. in culture and that *P. aphanidermatum* could not be recovered from water suspensions or soil to which both fungi had been added.

The points discussed above provide possible explanations for the difficulty encountered by Garren (4) and others (2, 13) in isolating certain fungi from pods which had symptoms typical of those suggestive of the respective fungal pathogens. *Pythium myriotylum,* in this study for example, was not isolated from rotted pods with typical symptoms associated with *P. myriotylum* when the pods were first exposed to *F. solani* or *T. viride* and then to *R. solani* and *P. myriotylum* in soil. Neither *P. myriotylum* nor *R. solani* could be isolated from attached pods exposed to *P. myriotylum* before *R. solani*.

Unless gnotobiotic conditions are used to evaluate interactions of fungi in peanut pods, *F. solani* and *T. viride* are consistently present as inhabitants of the endogeocarp or geocarposphere (2, 3, 4, 5, 13). When pathogenicity studies are conducted to evaluate fungi which are ubiquitously present in soil, it is essential to define clearly the inoculum levels prepared with reference to background populations of the respective fungi and relate these factors to the final isolations of fungi from pods.

Although there is a possibility of using avirulent isolates of *R. solani* to protect peanut pods from damage by *P. myriotylum,* the specific environmental conditions under which *P. myriotylum* is biologically controlled, but under which pods are not damaged by *R. solani,* must first be ascertained. Wills and Moore (18) have reported several isolates of *R. solani* from pods that were nonpathogenic to peanut seedlings, but no reports on the range of pathogenicity of isolates of *R. solani* to pods were found by the authors.

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