Synergistic Interactions of Pythium myriotylum with Fusarium solani and Meloidogyne arenaria in Pod Rot of Peanut

R. Garcia and D. J. Mitchell

Research Assistant and Assistant Professor, respectively, Department of Plant Pathology, University of Florida, Gainesville 32611.

Present address of senior author: Colégio Superior de Agricultura Tropical, Tabasco, México.

Supported in part by the Mexican Institution, Consejo Nacional de Ciencia y Technologia.

Florida Agricultural Experiment Station Journal Series Paper No. 5746.

ABSTRACT

Peanut pod rot was more severe when pods were exposed to soil containing combinations of *Pythium myriotylum* and *Fusarium solani* or *Meloidogyne arenaria* than when pods were exposed to *P. myriotylum* alone. Only *P. myriotylum* alone caused significantly more pod rot than that observed in the controls.

Phytopathology 65:832-833

Additional key words: inoculum density, *Arachis hypogaea*, peanut pod breakdown.

Pythium myriotylum Drechs. is the major cause of peanut pod rot (2, 3, 4, 7), but the disease may be greatly enhanced when other organisms such as soil mites (1), southern corn rootworm larvae (7), or various soil-borne fungi (2, 3, 4) also are present. *Pythium myriotylum, Fusarium solani* (Mart.) App. & Wr. emend Snyd. & Hans. and *Meloidogyne arenaria* (Neal) Chitwood were consistently associated with galled and rotted peanut fruits on plants grown on a farm with a high incidence of pod rot in 1972 in Levy County, Florida (3). Since a synergistic interaction with two or three of these organisms seemed possible, the present work was undertaken to determine the influence of *F. solani* and *M. arenaria* when combined with *P. myriotylum* at specific inoculum densities on peanut pod rot.

The fungi used in this investigation were isolated from rotted peanut pods, and hyphal-tipped cultures 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 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 g $CaCO_3$, and 900 ml distilled water). The flasks were maintained without shaking at approximately 25 C under continuous light for 15 days.

Arredondo fine sand was infested by mixing aseptically eight V-8 juice broth cultures of each fungus into 1,200 g of autoclaved soil with an electric hand-held mixer. Sterile distilled water was added to the soil to give a final water content of 10% (w/w), and the infested soil was maintained at approximately 27 C for 15 days in 2-liter Mason jars. Soil populations of *P. myriotylum* were assayed with the pimaricin-vancomycin selective medium ($P_{10}V_{100}$) of Tsao and Ocana (9), and soil populations of *F. solani* were assayed with the Tergitol NPX (1 ml/liter) and streptomycin sulfate (50 mg/liter) - amended potato-dextrose agar (PDA) by the method of Steiner and Watson (8). Infested soil was diluted with noninfested autoclaved soil to give 10 propagules of *P. myriotylum* per g of soil and 3,000 propagules of *F. solani* per g of soil.

Eggs of *M. arenaria* were extracted from galled peanut roots and the nematode was maintained on tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'). After the collection of egg masses from the tomato roots and the extraction of the eggs from the egg masses by the method of McClure et al. (5), the eggs were counted and added to soil immediately before use at the rate of 5 eggs/g of soil.

Twenty-six peanut pegs (gynophores) attached to mature plants grown in 20-liter cans (3) were used for each treatment. The pegs were surface disinfested for 40 seconds with 1.0% sodium hypochlorite, rinsed with sterile distilled water, and introduced into 50-ml test tubes containing soil infested with each pathogen alone or in combination with one or both of the other pathogens. The

TABLE 1. Incidence of pod rot and frequency of isolation of fungi from attached peanut pods grown in test tubes containing soil with defined inoculum densities[x] of pathogens established by diluting infested soil with autoclaved soil

Treatment	Pod rot (%)	Frequency of isolation (%)	
		P. myriotylum	*F. solani*
Noninoculated	0.0 a[y]	...[z]	37.0 a
M. arenaria	0.0 a	...	52.0 b
F. solani	5.5 a	...	77.7 b
F. solani + *M. arenaria*	7.6 a	...	100.0 c
P. myriotylum	19.2 b	38.4 ab	53.8 b
P. myriotylum + *F. solani*	47.0 c	21.0 a	100.0 c
P. myriotylum + *M. arenaria*	50.0 c	54.0 b	22.7 a
P. myriotylum + *M. arenaria* + *F. solani*	46.0 c	26.9 ab	100.0 c

[x]The populations of pathogens were 10 propagules per gram (ppg) of soil of *Pythium myriotylum*; 3,000 ppg of *Fusarium solani*; and five eggs per gram of soil of *Meloidogyne arrenaria*.

[y]Any given pair of treatments (within vertical columns) followed by the same letter are not different, $P = 0.05$, when compared by the contingency chi-square test.

[z]No isolation attempted.

tubes were covered with Parafilm and aluminum foil and the plants and tubes were maintained for 9 weeks in a greenhouse at 26-37 C. The pods were then removed, the percentage of pod rot was estimated, and one half of each pod was plated on $P_{10}V_{100}$ agar for the detection of *P. myriotylum* and the other half was plated on lactic acid-acidified PDA (pH 4.0) for the detection of *F. solani*. The data presented in this paper are the means of two experiments replicated 26 times for each treatment.

Significant percentages of peanut pod rot occurred only in soil containing *P. myriotylum* (Table 1). Synergistic interactions in pod rot were observed when *P. myriotylum* was combined with *F. solani, M. arenaria,* or a combination of both pathogens. The synergistic interaction between *P. myriotylum* and *F. solani* has been reported previously by Frank (2), who suggested that other organisms may function similarly to *F. solani* in interaction with *P. myriotylum*. Other reports have since indicated that soil mites (1), and the southern corn rootworm (7) interact with *P. myriotylum* in pod rot of peanut. This, however, appears to be the first report of a synergistic interaction between *P. myriotylum* and *M. arenaria* in the pod rot of peanut.

Pythium myriotylum was isolated from 38% of the pods exposed to soil containing 10 propagules of this fungus alone per g of soil. This incidence of infection compares favorably with Mitchell's (6) observation of 34% infection of rye seedlings in soil containing 10 oospores of *P. myriotylum* per g of soil. The frequency of isolation of *P. myriotylum* was not significantly greater from pods exposed to combinations of *P. myriotylum* and other pathogens than it was from pods exposed to *P. myriotylum* alone. More *P. myriotylum* was isolated from pods exposed to a combination of *P. myriotylum* and *M. arenaria* than from pods exposed to *P. myriotylum* and *F. solani*.

Fusarium solani was isolated from pods in all treatments, but was isolated from a significantly greater percentage of pods that were exposed to combinations of *F. solani* plus *P. myriotylum* or *M. arenaria* than in pods exposed to *F. solani* alone. When pathogenicity studies are conducted to evaluate fungi, such as *F. solani,* which are ubiquitously present and can reinfest treated soil, it is important to define clearly the prepared inoculum levels with reference to the background population and to relate this factor to the final isolations from pods. Fluctuations in the populations of *F. solani* in autoclaved or nontreated soil also could greatly influence the results of studies on the pathogenicity of single pathogens such as *P. myriotylum*.

Phytopathology 65:833-834

LITERATURE CITED

1. BEUTE, M. K. 1974. A quantitative technique for the extraction of soil-inhabiting mites (Acarina) and springtails (Collembola) associated with pod rot of peanut. Phytopathology 64:571-572.
2. FRANK, Z. R. 1972. Pythium myriotylum and Fusarium solani as cofactors in a pod-rot complex of peanut. Phytopathology 62:1331-1334.
3. GARCIA, R. 1974. Interactions of Pythium myriotylum with Fusarium solani, Rhizoctonia solani and other fungi and with Meloidogyne arenaria in peanut pod rot and preemergence damping-off. Ph.D. Dissertation, Univ. Florida, Gainesville. 51 p.
4. GARREN, K. H. 1967. Relation of several pathogenic organisms, and the competition of Trichoderma viride to peanut pod breakdown. Plant Dis. Reptr. 51:601-605.
5. MC CLURE, T., H. KRUK, and I. MISAGHI. 1973. A method for obtaining quantities of clean Meloidogyne eggs. J. Nematol. 5:230.
6. MITCHELL, D. J. 1975. Density of Pythium myriotylum oospores in soil in relation to infection of rye. Phytopathology 65:570-575.
7. PORTER, D. M., and J. C. SMITH. 1974. Fungal colonization of peanut fruits as related to southern corn rootworm injury. Phytopathology 64:249-251.
8. STEINER, G. W., and R. D. WATSON. 1965. Use of surfactants in the soil dilution plate count method. Phytopathology 55:728-739.
9. TSAO, P. H., and G. OCANA. 1969. Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. Nature (Lond.) 223:636-638.