

**Pythium myriotylum and Fusarium solani as Cofactors in a
Pod-Rot Complex of Peanut**

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ABSTRACT

Pod rot of peanuts is caused by synergistic interaction of *Pythium myriotylum* with *Fusarium solani*. Unlike previously known pathogenic complexes of synergistic soil fungi, in the present case neither pathogen, when alone, is an effective pathogen of the involved host organ. *Pythium* is a latent endophyte of peanut pods; *Fusarium*, although frequently occurring in apparently healthy pods, rarely causes any symptoms. In previously sterilized soil, inoculation of pods with *Fusarium* followed by inoculation with *Pythium* yielded a significantly higher proportion of

diseased pods than did inoculation in the reverse order. It is inferred that *F. solani* predisposed pods to pathogenic activity of *P. myriotylum*. In naturally infested soil to which additional inocula were added, *Pythium* caused a high proportion of slightly rotted pods, whereas *Fusarium* caused a small proportion of severely rotted pods. Hence, *P. myriotylum* may be the principal cause of rot, whereas the predisposing *F. solani* is involved also in the final disintegration of diseased pods.

Additional key words: *Arachis hypogaea* synergism.

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Pod rot of peanut is prevalent in sandy soils of Israel. Previous work (6) showed that *Pythium* isolates from rotted peanut pods (later all identified as *P. myriotylum*) are causal agents of the rot. However, when *P. myriotylum* alone was added to sterilized soil, sheltered from heavy contamination by dust-borne inocula, no rot occurred, although *P. myriotylum* could be reisolated from a proportion of the pods.

Occasionally, *P. myriotylum* was the only fungus isolated from very young, almost imperceptibly diseased geocarps. However, from lignifying but still soft pods showing distinct rot symptoms, *Fusarium* spp. were isolated in addition to *P. myriotylum*. In disintegrating pods, collected from fields and inoculation experiments, the incidence of *Fusarium* spp., commonly *F. solani*, was higher than that of *Pythium* spp., which indicated that *F. solani* was a possible cofactor in causing the disease. Therefore, the roles of these fungi in pod rot development were studied.

MATERIALS AND METHODS.—Inocula of *Pythium myriotylum* Drechsler and *Fusarium solani* (Mart.) Appel & Wr. emend. Snyder & Hans. were produced on a 12:6:3 (v/v) sand-soil-oatflake mixture. Peanut plants (*Arachis hypogaea* L. 'Virginia Sihit Meshubahat', a standard commercial Israeli jumbo-type cultivar) were grown outdoors in large concrete containers (50 cm inner diam). I inoculated pods by infesting the soil during the fruiting period up to approximately the middle of the pods' development period. Development of the individual pod lasts about 9 weeks from pollination to maturity; the reproductive phase of the plant lasts about 13 weeks.

In the first experiment, the gynophores were allowed to grow into soil which was initially dry-heat sterilized (130 C for 6 hr), contained in 17-cm-high porous clay pots with sealed bottoms (Fig. 1). Except in the case of controls, the soil was later replaced by inocula, diluted 1:4 (v/v), with sterile soil. The treatments applied were: (i) noninoculated, initially sterile control; (ii) *F. solani* only, added 2 weeks after the first gynophores penetrated the soil; (iii) *P. myriotylum* only, at the same stage of pod development; (iv) beginning as in treatment No. 2, but with the removal of *Fusarium*-infested soil 1 month later and its replacement by *Pythium* inoculum; (v) beginning like treatment No. 3, but with the removal of *Pythium*-infested soil a month later and its replacement by *Fusarium* inoculum; and (vi) simultaneous soil infestation with *Pythium* and *Fusarium* 6 weeks after the first gynophores invaded the soil (i.e., the date of inoculum replacement in treatments 4 and 5).

The pods were harvested 1 month after the last inoculations; i.e., 10 weeks from the beginning of fruit-setting. They were rated for the presence and severity of rot, and were sorted into soft-unlignified and lignified fruits. The entire yield from treatment-replicates producing few pods, or representative samples of pods where yields were larger, were surface-sterilized with 1% NaOCl, and plated on selective media modified after Garren (7) and Eckert & Tsao (2). One-half of each pod was

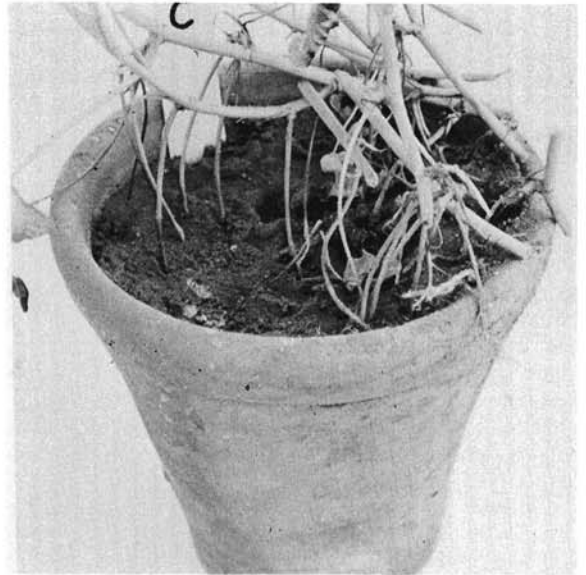


Fig. 1. Method of growing and inoculating pods in previously sterilized clay pots. Peanut plants are grown in large containers. Gynophores from side branches are allowed to grow into adjacent clay pots (as shown) used for inoculation. Gynophores initially develop pods in heat-sterilized soil, which is later replaced by inoculum diluted with heat-sterilized soil.

plated on 1.5% tap water agar (TWA) amended with 20 μ g/ml rose bengal and 50 μ g/ml streptomycin to isolate *Fusarium* spp., and various other fungi and bacteria. This medium retarded the growth of some *P. myriotylum* isolates. To isolate *Pythium* spp. successfully, the other half of each pod was plated on TWA amended with 0.17% Difco's cornmeal agar, 100 μ g/ml pimarinic, 50 μ g/ml bacitracin, and 50 μ g/ml crystalline penicillin.

The experiment was laid out in six randomized blocks. Because of the small and unequal number of pods produced in the various treatment replicates, percentages of diseased pods were based on small and variable denominators. The sign-test was thus applied in preference to the standard analysis of variance. An interaction of *Fusarium* with *Pythium* could be verified, by approximation, using the t-test.

In the second experiment, large concrete containers (50 cm diam, 80-130 cm height), placed in fixed position, were classified according to equal height in groups of three. These containers of sandy nonsterilized soil were randomly contaminated with pod rot organisms, the degree of contamination being low but varying between containers. Because of this variability, no reliable controls could be established. Therefore, excess-inoculum treatments were compared with each other: 2 liters of inoculum/container of either *P. myriotylum* or *F. solani*, or a 1:1 mixture of the two, were placed beneath the gynophores at the onset of fruit set. At harvest, the severity of rot in each affected pod was rated on a 1-4 scale with 1 = slight symptoms and 4 = disintegration, and treat-

ment means for entire yields and for diseased pods only were determined. The percentage of diseased pods was also computed. The nonrandomized experiment was replicated 5 times and analyzed by the χ^2 yrdy.

RESULTS.—Treatment effects in sterilized soil (Table 1) merit detailed consideration. *Fusarium* spp., most of them *F. solani*, were isolated from surface-sterilized pods in the controls and the *Pythium* treatment (treatments 1 and 3). It was noted that the *Fusarium* spp. proliferated in the *Pythium* treatment, possibly due to the food-base of the artificial *Pythium* inoculum. Inoculation with *F. solani* alone caused a very low incidence of diseased pods, although the fungus was frequently reisolated from pods. A high frequency of diseased pods resulted only when *Pythium* was present (Table 1, treatments 3-6); nevertheless, *Pythium* could not be reisolated from every pod with obvious disease. Only the sequence of inoculation first with *F. solani*, then with *P. myriotylum* (treatment 4), produced a significantly higher incidence of rotted pods than the sum of effects of separate *Fusarium* and *Pythium* treatments ($P = .01$). This confirms the existence of a synergistic interaction. This sequence of inoculations also resulted in a significantly higher proportion of rotted pods than that caused by the reverse order of inoculation ($P = .03$). It is inferred that *F. solani* predisposes the pod to rot caused by *P. myriotylum*. Consequently, *P. myriotylum* is regarded as the principal causal organism.

TABLE 1. The effects of infesting initially sterile soil with *Pythium myriotylum* and *Fusarium solani* on the frequency of peanut pods from which *Fusarium* and/or *Pythium* were isolated, and on the incidence of pod rot

Treatment	% Pods yielding ^a		% Rotted pods
	<i>Fusarium</i>	<i>Pythium</i>	
1. Control ^b	29.0 a	0	2.3 a
2. <i>F. solani</i>	69.7 b	0	6.4 a
3. <i>P. myriotylum</i>	68.9 b	16.1 ab	30.9 b
4. <i>F. solani</i> → <i>P. myriotylum</i> ^c	58.1 b	31.1 b	53.7 c
5. <i>P. myriotylum</i> → <i>F. solani</i> ^c	68.9 b	8.1 a	35.6 b
6. <i>P. myriotylum</i> + <i>F. solani</i> ^d	55.0 b	9.1 ab	39.2 bc

^aMeans of six replicates. Within any one column, values not followed by the same letter differ significantly. Analysis by sign-test ($P \leq .0625$; in most comparisons $P = .03125$).

^bDust-borne *Fusarium* spp. inocula involved, mainly *F. solani*.

^cDifferent inocula were successively added to the same soil at a 4-week interval.

^d'Simultaneous' soil infestation with both fungi on the later date.

In the second experiment (Table 2), where unsterilized soil already infested with both rot organisms was used, infestation with additional inoculum of *P. myriotylum* resulted in a significantly high proportion of slightly to moderately diseased pods. *Pythium myriotylum* is therefore regarded as the direct cause

TABLE 2. Effect of adding, to naturally infested soil, inocula of *Pythium myriotylum* alone, *Fusarium solani* alone, or a 1:1 mixture of the two, on the frequency and severity of peanut pod rot

Treatment	Mean severity index in ^a		Prevalence of rotted pods (%)
	Whole yield	Rotted pods	
<i>Pythium</i> alone	0.88	1.99 B	43.2 a
<i>Fusarium</i> alone	0.64	2.96 A	23.1 b
<i>Pythium</i> + <i>Fusarium</i> at half volume each	0.73	1.33 B	45.0 a

^aMeans of five prefixed replicates; analysis by χ^2 test. Values not followed by the same letter differ significantly (lower case letters: $P = .05$; upper case letters: $P = .005$).

of initiation of disease symptoms. In the same soil, adding a heavy inoculum of *F. solani* affected only a small proportion of pods, although these were severely rotted. It is inferred that *F. solani* intensifies or accelerates final disintegration of diseased pods. Thus, the results of the two experiments are complementary.

DISCUSSION.—*Fusarium solani* and other *Fusarium* spp. are components of the common microflora of peanut pods (8, 9, 10, 12), whereas *Pythium myriotylum*, whether latent or active, is unique to pods in rot-affected field plots (7). However, *P. myriotylum* alone does not cause pod rot (6). In the present work, *F. solani* was found to influence disease both by predisposition to *P. myriotylum* and by contributing to disintegration after infection by *Pythium*. Some other, less prevalent, members of the pod microflora might possibly function similarly to *F. solani*. In the present experiments as well as in the field, *Pythium* was often absent from rotted pods in the late phase of decay, as if displaced by *F. solani* and by other fungi and bacteria which could be isolated from the pods. Apparently, these unspecified microorganisms are saprophytically involved in the rot.

A summary of the interrelation of microbial effects in the etiology of this pod rot is shown in the following scheme:

Phase I	Phase II	Phase III
<i>Fusarium</i> predisposition (<i>Pythium</i> sporadic)	<i>Pythium</i> necrosis (<i>Pythium</i> frequent)	<i>Fusarium</i> and saprobes disintegration (<i>Pythium</i> disappears)

Synergism among soil-borne pathogenic fungi has been investigated, but only rarely (5). Fawcett (4) found that a saprophytic *Fusarium* sp. infected lesions which had been caused by *Phytophthora citrophthora* on Citrus trunks, and aggravated the disease. Kerr (11) found that *F. oxysporum* and *P. ultimum*, both pathogens on their own, interact positively in causing much more severe pea wilt than the additive effect of the separate diseases. Elarosi (3) found that the succession of *Rhizoctonia solani*

preceding *F. solani* not only aggravates dry rot of potato, but also causes qualitative histopathological changes. Alconero & Santiago (1) showed that *R. solani*, the relatively harmless endomycorrhizal fungus of *Vanilla phaeanta*, predisposes the plant to invasion by *F. oxysporum* f. sp. *vanillae*. Subsequently, this *Fusarium* upsets the symbiotic equilibrium, thus converting the interacting fungi into a highly pathogenic complex. I, too, noted the existence of a fungus, *F. solani*, which rarely causes damage to pods by itself, but which predisposes them to *Pythium*. In contrast to the above-mentioned example in *Vanilla*, however, no distinction can be made in peanut pod rot between the primary and the secondary parasite, since each of the two fungi can invade the pod and exist there latently. Also, it is not clear which of the two fungi involved is the primary pathogen and which is the secondary since: (i) The typical and common damage is not caused by each parasite alone but by the pathogenic complex of both; (ii) although *F. solani* predisposes the pods to the activity of *P. myriotylum* (not vice versa), this *Pythium* is the decisive factor in symptom initiation; and (iii) *F. solani* outlives the *Pythium* hyphae and engages in further pathogenic activity. Thus, the synergism which causes the *Pythium*-complex pod rot differs in some aspects from synergistic interactions known previously.

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