Evidence for the Involvement of Soilborne Mites in Pythium Pod Rot of Peanut

H. D. Shew and M. K. Beute

Graduate Research Assistant and Professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh, 27650.

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ABSTRACT

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Mites of the genus *Caloglyphus* (Acarina: Acaridae) were associated with more than 50% of decaying peanut pods collected in a field in which pod rot was caused by *Pythium myriotylum*. Laboratory cultures of these mites could be maintained for several months on *Pythium aphanidermatum* growing on potato-dextrose agar slant tubes. In food preference tests *Caloglyphus micheali* was attracted to *P. myriotylum*; up to 98% of all mites responding in food preference tests preferred *P. myriotylum* over five other fungi isolated from peanut pods. Viable colonies of *P. myriotylum* were obtained from 90% of the fecal pellets collected from *C. micheali* after feeding on mycelial mats of the fungus. *P. myriotylum* oospores also remained viable after passing through the alimentary canal of *C. micheali*. Pythium pod rot was reduced significantly in field and greenhouse tests of several acaricides and broad spectrum insecticides. In greenhouse tests the addition of soilborne mites to field soil infested with *P. myriotylum* significantly increased the incidence of peanut pod rot.

Additional key words: *Pythium myriotylum*, *Caloglyphus* spp.

Although mites are the most numerous of all soilborne arthropods, only limited research has been conducted to investigate the role of this group of organisms in the initiation of fungal (4,16) and bacterial (6) plant diseases. Several reports of mites as pests of peanut (*Arachis hypogaea* L.) are known. *Caloglyphus rodionovi* Zach. was capable of penetrating the peanut pod and feeding on the developing kernels (21). Mites of the genera *Caloglyphus* and *Tyrophagus* were isolated regularly from subterranean parts of peanut plants in South Africa and under certain conditions penetrated pods and increased percent colonization of these pods by *Aspergillus flavus* (Link) Fr. (1).

Enhancement of peanut pod rot caused by *Pythium myriotylum* Drechs. has been reported in the presence of various soil flora (7,10) and fauna (9,18) including soil mites (2,3). This study was undertaken to determine: (i) which mite species are associated with rotting pods in North Carolina; (ii) if mites enhance Pythium pod rot development; and (iii) the possible mechanisms of mite-associated disease enhancement.

MATERIALS AND METHODS

Isolates of *P. myriotylum* and all other fungi used in this study were isolated originally from peanut pods or plants. Stock cultures were maintained on glucose yeast-extract agar or potato-dextrose agar (PDA) at 24-26 C. Soilborne mites were obtained from peanut pods decayed by *P. myriotylum*, pods that had overwintered in field soil, and from soil collected in a field with a history of Pythium pod rot. Mites were extracted from soil by a sugar flotation-sieving technique (3).

Mite culture and identification. Following initial isolation of mites from peanut pods and soil, mites were placed on agar slant tube cultures of *Pythium aphanidermatum* (Edson) Fitzp. growing on PDA at 24-30 C. New colonies were started by transferring 5-10 gravid females to a 5-day-old tube culture of *P. aphanidermatum*. Only adult mites were used in in vitro experiments.

For mite identification, a single gravid female from each mite isolate was transferred to a new culture tube to ensure pure cultures of mites for future work. Mites used for identification were mounted in Hoyer's solution (15). Identification was made by M. H. Farrier, Entomology Department, North Carolina State University, Raleigh, and by the authors.

Pod rot control and enhancement. A field test was conducted to evaluate the effectiveness of several acaricides and broad spectrum insecticides in reducing pod rot incidence and severity. The field used had a history of Pythium pod rot. Seeds of peanut cultivar VA 56 R were planted in early May and all standard agronomic practices were observed throughout the growing season except for application of test insecticides and acaricides. These chemicals were applied at a rate of 2.24 kg AI/ha as a drench in a 30 cm band over the row in mid July using approximately 655 L H_2O/ha to apply the chemicals. Pod-rot data were collected the last of September. Plots consisted of two 15.2 m rows and were replicated four times in a randomized complete block design.

The soil used in greenhouse tests was Norfolk fine sandy loam taken from the plow layer (top 18 cm) of the field described above. Cultivar NC 5 peanut plants were grown in containers 50 × 35 × 14 cm. Chemicals were applied as a drench at equivalent rates described for field tests. After 16 wk plants were rated for pod rot and mite populations were determined using the sugar flotation-sieving method (3).

Pod rot enhancement tests were conducted on NC 5 peanut plants grown in sterilized fine sandy loam soil in 50 × 35 × 14-cm containers in the greenhouse at 24–30 C. Treatments included infesting soil with (i) *P. myriotylum* plus mites, (ii) *P. myriotylum* alone, (iii) mites alone, and (iv) no treatment (controls). Fungal inoculum was introduced by placing five oat grains, inoculated 10 days prior with *P. myriotylum*, into each corner of the containers approximately 2.5 cm below the soil surface. Sterile oat grains were substituted in treatments not receiving *P. myriotylum*. Mites were introduced as a mixture of isolates onto the oat grains. Approximately five living mites were placed in each corner of the container. The soil was infested when the plants were 10 wk of age, the test ran for an additional 10 wk, and the test was repeated three times.

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Feeding preference, attraction, and fungus transmission. Laboratory tests were conducted to determine the feeding preference of *Caloglyphus micheali* for various fungal species. The fungi used in food preference tests were: *Aspergillus flavus*, *Rhizoctonia solani* Kuhn, *Trichoderma viride* Pers. ex Fr., *Sclerotium rolfsii* Sacc., a *Penicillium* sp. and *Pythium myriotylum*. All fungi were isolated from diseased peanut pods. Nine-millimeter diameter plugs of 5-day-old fungal cultures grown on PDA at 24 C were placed in various combinations in 9-cm diameter petri dishes. Mites were washed in 0.5% sodium hypochlorite for 1 min, and then introduced into the petri dishes on 4.25-cm-diameter filter paper disks positioned equidistant from test plugs. The petri dishes were placed in desiccators in which a constant relative humidity of 85% was maintained by a saturated potassium chloride solution (22). The number of mites on each test plug was counted at 2, 6, 12, 24, and 48 hr. The experiment was repeated twice with similar results.

Prior research (1,11) has demonstrated that some Acaridae mites exhibit a strong attraction to a source of moisture. Petri dish tests were conducted to observe the Acaridae mites isolated from peanut pods for a similar response. A 9-mm-diameter plug of 2% water agar (WA) placed in a 9-cm diameter petri dish served as a moisture source. A single adult mite was introduced into the petri dish at various distances from the agar plug. The petri dish was placed over grid paper to facilitate monitoring of mite movements.

The in vitro transmission of *P. myriotylum*; ie, ingestion and passing of viable fungal propagules, was tested. Mycelial mats of the fungus were grown in 12 ml of potato-dextrose broth in a 250-ml flask for 2 days at 30 C. The mycelial mat was then removed, washed twice in sterile distilled water and placed in a sterile petri dish. Fruiting structures were not observed on the mycelium. Ten mites were introduced onto the fungus mat with a fine transfer needle. After 24 hr of feeding, mites were collected, rinsed in 0.5% sodium hypochlorite and placed on a modified triple-P (pimaricin, 10 ppm; penicillin-G, 50 ppm; and polymixin-B, 50 ppm) agar medium (5). Fecal pellets deposited by mites on the agar surface were collected and transferred to new plates of medium. Plates were incubated at 27 C and checked for growth of *P. myriotylum* from fecal pellets at 24-hr intervals.

Oospores for consumption by mites were produced by placing a 4-cm diameter plug of oatmeal agar in the center of a 9-cm diameter petri dish and transferring a 4-mm diameter plug cut from a culture of *P. myriotylum* grown on PDA onto it. After incubation for 2 days at 30 C the plate was flooded with 0.3% WA. Oospores were produced in abundance in the WA with a minimum of mycelium after 3–4 days. Plugs of the WA containing oospores were placed in sterile petri dishes and surface-disinfested mites were introduced. The procedure for collection and plating of mite fecal pellets was repeated.

RESULTS

Mite culture and identification. Acarid mites were cultured easily on *P. aphanidermatum* PDA slant tube cultures. The rapid growth rate and abundant aerial mycelium produced by *P. aphanidermatum* on that medium was ideal for growth and reproduction by the acarid mite isolates. In food preference tests *P. aphanidermatum* was equally as attractive to the mites as was *P. myriotylum*. This strong attraction and its nonpathogenicity to peanut, led to the selection of that fungus species for all mite culturing in these experiments.

Mites of the suborders Mesostigmata, Astigmata, and Cryptostigmata, were isolated from field soil and decaying peanut pods. Only members of the Astigmata were found associated with a high percentage (>50%) of diseased pods. These mites also were isolated from the root and pod zone of healthy peanut plants. Three acarid mite species were identified. All isolates were of the genus Caloglyphus, and one isolate was identified as *C. micheali*.

Pod rot control and its enhancement. In field tests all treatments except diazinon reduced ($P = 0.05$) peanut pod rot (Table 1). No differences in control of pod rot were detected among the remaining chemicals. Greenhouse tests demonstrated that not all acaricides and insecticides reduced pod rot. In the first test aramite, dicofol, and the nematicide-insecticide ethoprop did not reduce pod rot compared to untreated control plants (Table 2). In the second test diazinon, which was not effective in field tests, reduced pod rot in the greenhouse (Table 3). In greenhouse tests pod rot reduction was correlated with a reduced mite population in all cases except those for dimethoate (Table 2) and monocrotophos (Table 3). The chemicals propargite and carbofuran, which reduced pod rot incidence in each of the tests, were not inhibitory to *P. myriotylum* when incorporated in PDA at the rates used in tests.

TABLE 1. Peanut pod rot incidence in a field test after acaricide and insecticide treatment

Treatment[y]	Pod rot[z] (%)
Propargite	6 a
Cyhexatin	13 a
Aldicarb	7 a
Disulfoton	0 a
Chlordimeform	2 a
Monocrotophos	4 a
Fonofos	11 a
Carbofuran	14 a
Diazinon	60 b
Control	46 b

[y] Chemicals were applied at the rate of 2.24 kg AI/ha as a drench in a 30 cm band over the row using approximately 655 L of H_2O/ha.
[z] Values followed by the same letter are not significantly different, $P = 0.05$.

TABLE 2. Effect of chemicals on incidence of peanut pod rot and control of soil mites in field soil in the greenhouse

Treatment[x]	Pod rot (%)	Mites/50cc soil[y] (no.)
Propargite	0 a[z]	0 e
Carbofuran	0 a	0 e
Aldicarb	4 b	1 e
Dimethoate	7 b	9 f
Oxythioquinox	8 b	0 e
Aramite	14 cd	17 f
Dicofol	14 cd	16 f
Ethoprop	14 cd	13 f
Control	27 d	13 f

[x] Chemicals were applied at the rate of 2.24 kg AI/ha as a drench using aproximately 655 L of H_2O/ha.
[y] The average of four determinations.
[z] Values followed by the same letter are not significantly different, $P = 0.05$.

TABLE 3. Relationship between control of soil mites and peanut pod rot in field soil in a greenhouse test

Treatment[x]	Pod rot (%)	Mites/50cc soil[y] (no.)
Propargite	0.4 a[z]	1.2 c
Disulfoton	0 a	0.5 c
Carbofuran	11.2 a	0.3 c
Diazinon	0 a	0 c
Monocrotophos	4.5 a	2.2 d
Cyhexatin	3.0 a	1.8 c
Chlordimeform	6.7 a	0.5 c
Fonofos	1.3 a	1.2 c
Control	44.4 b	5.0 d

[x] Chemicals were applied at the rate of 2.24 kg AI/ha as a drench using approximately 655 L of H_2O/ha.
[y] Average value of four determinations.
[z] Values followed by the same letter are not significantly different, $P = 0.05$.

When data from three greenhouse tests were combined for analysis, pod rot incidence increased ($P = 0.05$) when both *P. myriotylum* and soilborne mites were present (77.3%) compared to treatments receiving the fungus (47%) or mites (12%) alone (Table 4). There was no pod rot in the control plants. Disease enhancement was observed only when a mixture of mite species was used: *Oppia* sp. or *C. micheali* alone failed to enhance disease. However, a trend toward disease enhancement was observed for the *C. micheali* mite isolate (20).

Mite feeding preference, attraction, and fungus transmission. In all feeding preference tests mites were attracted to *P. myriotylum* more than to *R. solani*, *A. flavus*, *T. viride*, *S. rolfsii*, or *Penicillium* sp. (Table 5). When tested singly or in combination with all the above fungi up to 98% of the total number of mites which responded to any food source were attracted to *P. myriotylum*. More mites were attracted to *T. viride* than to other fungi except for *P. myriotylum* when all fungal species were tested at once. Only a few mites were attracted to uninoculated WA and PDA plug controls.

C. micheali adults exhibited a definite klinotactic response to the WA plug after 2–10 min (depending on placement of mites 2–5 cm away from the plug) of random movement. In food preference tests mites did not leave WA plugs unless plugs were present that provided both food and moisture. Similar mite behavior was observed in two additional tests involving 40 adult mites. A WA plug, a PDA plug containing *P. myriotylum*, and several peanut pods were placed in a petri dish and the mites were introduced on a moist filter paper disk. As the filter paper disk dried, mites migrated to various points in the plate; approximately 15% were killed during transfer from tubes to the filter paper disk, 60% responded to the *P. myriotylum* plug, 26% to the mature peanut pod, 7% to the immature pod, and 7% to the WA plug.

Mites ingested and passed viable propagules of *P. myriotylum*. The fungus grew from 90% of the boli collected from mites which had fed on *P. myriotylum* mycelial mats. Mites placed on WA containing oospores often selectively fed around the oospores and on the sparse mycelium that was present. Approximately 5% of the boli collected and examined microscopically contained oospores, while approximately 1% of the boli plated on TP media gave rise to colonies of *P. myriotylum*. Oospores were found infrequently in boli collected from adult mites that had fed on artificially inoculated pods in pasteurized soil in the laboratory.

TABLE 4. Enhancement of peanut pod rot in greenhouse tests by simultaneous addition of a mixed culture of mites and *Pythium myriotylum*

Treatment	Pod rot[y] (%)
Mites alone	12 a[z]
P. myriotylum alone	47 b
Mites plus *P. myriotylum*	77.3 c
Control	0 a
LSD = 29.9	

[y] Values given are the combined average of three tests.
[z] Values followed by the same letter are not significantly different, $P = 0.05$.

TABLE 5. Feeding preference of *Caloglyphus micheali* (soilborne mites) for *Pythium myriotylum* compared to other fungi as determined in petri dish tests

Fungus species[v]	Mites per plug[y,z] feed time				
	2 hr (no.)	6 hr (no.)	12 hr (no.)	24 hr (no.)	48 hr (no.)
Pythium myriotylum[w]	29	36	42	54	44 a
Aspergillus flavus	22	12	8	5	3 b
Pythium myriotylum	25	40	41	41	44 a
Trichoderma viride	26	19	13	16	8 b
Pythium myriotylum	24	49	52	47	49 a
Rhizoctonia solani	3	6	5	7	1 b
Pythium myriotylum	23	37	38	34	35 a
Potato dextrose agar	17	9	9	10	6 b
Pythium myriotylum[x]	37	51	53	59 a	...
Water agar	1	3	3	3 c	...
Trichoderma viride	29	19	14	10 b	...
Potato dextrose agar	1	3	2	3 c	...
Sclerotium rolfsii	1	1	5	1 c	...
Aspergillus flavus	13	10	6	2 c	...

[v] Fungi were introduced as 9-mm diameter plugs of 5-day-old PDA cultures.
[w] Test involved only *P. myriotylum* and one other fungus.
[x] Test included four fungi species and WA and PDA checks.
[y] Data presented are averages of two tests, four replicates each.
[z] Paired numbers followed by the same letter are not significantly different, $P = 0.05$.

DISCUSSION

Frequently, Caloglyphus mites were isolated from decaying peanut pods collected from a field with a history of Pythium pod rot. Mites of that genus have been reported as a pest of peanut pods in both South Africa (1) and Czechoslovakia (21). In these reports mites penetrated peanut pods and introduced fungi into the pod and fed upon the already decaying kernels. Mites representing the families Ascidae, Anoetidae, and Oppidae also were isolated from decaying peanut pods but less frequently and in fewer numbers than were Caloglyphus mites. A similar complex of mites was reported to be present in diseased gladiolus corms in Florida (17).

Mite food preference has been assessed in several ways (12). The most commonly encountered and perhaps the easiest method used is to determine which food source attracts the most mites per unit time. Since Caloglyphus mites prefer fungi as a food source (13) various species of fungi were tested as food sources by the attraction method. In repeated tests *C. micheali* preferred *P. myriotylum* over other fungi tested. This preference may be important in mite ingestion of *P. myriotylum* propagules located in soil and in diseased peanut pods. Hyphal fragments and oospores of *P. myriotylum* pass through the alimentary canal of *C. micheali* and remain viable, thus allowing dissemination of the pathogen. These results agree with reports of similar relationships between acarid mites and certain fungi (11,19).

Peanut pods are susceptible to *P. myriotylum* rot during the entire developmental period (14,20). It also is reported that the surface of developing peanut pods remains moist even when the surrounding soil is dry (8). Caloglyphus mites are attracted to a moisture source (1,11,20) and to peanut pods in soil (1), and also may disseminate *P. myriotylum* propagules. Soilborne mites gather around *P. myriotylum*-infected peanut pods in soil and, subsequent to total decay and desiccation of rotted pods, probably enhance pathogen spread to adjacent healthy pods and introduce propagules of *P. myriotylum* to the peanut pod surface or interior.

Indirect evidence of the involvement of soilborne mites in peanut pod rot is provided by reduced incidence in acaricide-treated field and greenhouse test plots. Although reduced pod rot in field plots could not be attributed soley to mite control, there was a consistent positive correlation between reduced mite population and reduced pod rot in greenhouse tests. Disease enhancement also was observed when both mites and *P. myriotylum* were introduced into soil, compared to introduction of the fungus or mites alone. Although *P. aphanidermatum*, which was used to rear mites, did not cause peanut pod rot in previous tests (M. Beute, *unpublished*), it is possible that the low incidence of pod rot in the mites-alone treatment may have resulted from a mite-*P. aphanidermatum* interaction. This suggestion is supported further by the absence of pod rot in the control plants.

This study differs from previous work on the interaction of soil fauna and *P. myriotylum* (9,18) in that unlike the root knot nematode and southern corn rootworm, soilborne mites are not usually pests of peanut plants. Thus, the ecological and biological interactions involved in this system are difficult to define. However,

we feel that the primary role of mites in this disease is as a disseminating and not a wounding agent. This conclusion is based on results of the disease enhancement tests in which the mites moved outward from four point sources of inoculum, and on the biology of the mites involved; ie, they are mycophagus and do not feed on healthy plant tissue. The cosmopolitan nature of Acaridae mites and their close association with microbial populations warrant further consideration of them in studies of soilborne disease complexes.

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