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NEMATODES AND FUNGI ASSOCIATED WITH POD ROT OF PEANUTS IN OKLAHOMA

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Summary. Peanut fields in four Oklahoma counties were surveyed for pod rot and associated plant parasitic nematodes from early September to mid October during 1983-1985. Mean annual pod rot incidence over 3 years was 5.8-21.3%. *Meloidogyne hapla* was the most commonly found plant-parasitic nematode, followed by *Pratylenchus brachyurus*, and species of *Tylenchorhynchus* and *Criconemella*. Pod rot incidence (15.1%) was greater (P = 0.05) in fields infested with these nematodes than in fields without them (7.1%). *Meloidogyne hapla* was consistently associated with presence of pod rot in fields, and with fungi isolated from pods. *Pythium myriotylum*, *Rhizoctonia solani* (anastomosis group 4) *Sclerotium rolfsii* and *Fusarium* spp. were the principal fungi isolated from pods with symptoms. Survey results suggest that plant parasitic nematodes, particularly *M. hapla*, play an important role in peanut pod rot etiology in Oklahoma.

Peanut (*Arachis hypogaea* L.) is a protein rich crop of great importance in many tropical countries. Production in the United States is limited to several Southern states, one of which is Oklahoma, where the crop is valued at $60-70 million annually. Pod rot of peanut is a soilborne disease that can limit peanut production. Pod rot occurs worldwide (Frezzi, 1956; Kranz and Pucci, 1963; Frank, 1972; Ibrahim *et al.*, 1977; Mercer, 1977; Filonow *et al.*, 1988). Infected pods show various degrees of discoloration, from superficial russeting to complete blackening of the hulls, plus various stages of hull and kernel decay. Pegs may be infected and the junction between peg and pod is so weakened that substantial loss of pods occurs at harvest.

The disease is usually considered to be of complex etiology. Nutrient imbalances, particularly calcium deficiencies in some soils have been implicated in pod rot etiology (Csinos *et al.*, 1984). Fungi, either acting alone or in combination, have been reported to cause pod rot. Of these, *Pythium myriotylum* Drechs. (Garren, 1970; Frank, 1972; Filonow *et al.*, 1988), *Rhizoctonia solani* Kuehn (Garren, 1970; Filonow *et al.*, 1988) and *Fusarium solani* (Mart.) Appel. *et* Wr. Emend Snyd. *et* Hans. (Frank, 1972) have received the major attention. *Sclerotium rolfsii* Sacc. is a potential pod-rotting fungus (Ashworth *et al.*, 1961) that has received minimal attention.

Significantly more pod rot occurred in peanuts grown in soil infested with *Meloidogyne arenaria* (Neal) Chitwood and pod-rotting fungi than soil infested with the fungi alone (Garcia and Mitchell, 1975). Fungal invasion of pods was also increased by the presence of *Pratylenchus* species (Minton and Jackson, 1969). The presence of other plant-parasitic nematodes such as *Criconemella* spp. has been reported in peanut fields (Motsinger *et al.*, 1976; Ingram and Rodriguez-Kabana, 1980), but their involvement in the development of a pod rot complex has not been investigated.

This study was initiated to survey major peanut growing areas in Oklahoma and to identify plant parasitic nematodes and fungi that influence the incidence of peanut pod rot.

Materials and methods

Surveys were conducted from September to mid October each year during 1983-1985. Peanuts fields in Caddo, Bryan, Hughes and Okfuskee counties were sampled. Fields were visited once during the year and twelve plants per ha (5-20 ha per field) were randomly selected by walking 10 strides (ca. 9m) up a row, sampling, and then striding 5 rows over (ca. 4.5m) and up the 5th row for 10 strides to sample again. This procedure was continued up and down a field to give an inverted V or W sampling pattern. At each sampling site three plants were removed from the soil and observed for pod rot symptoms. Fields with little or no visible pod rot were sampled more intensively. All plants with symptoms of pod rot and a few without symptoms were collected. Soil samples were collected from the pegging zones of plants at each sampling site. Pods were washed in water and reinspected for pod rot symptoms. Pod rot was verified by the presence of characteristic symptoms and isolation of *P. myriotylum* or *R. solani* [anastomosis group (AG) 4] (Filonow *et al.*, 1988).

Soil samples were stored 3-7 days at 10° C until processed. Companion soil samples from each field were mixed well and 100 cc subsamples were processed for nematode extraction using a modification of the Christie-Perry technique (Christie and Perry, 1951). Roots were processed for nematode determinations within 1-2 days after collection. Lateral roots of each plant were cut into 1-2 cm pieces and 1 g subsamples incubated in aerated water for 48 h to extract plant parasitic nematodes (Russell, 1987). Nematodes were identified and their population densities determined.

Fungi were isolated from a 500 ml subsample of pods collected from each field. Hull pieces of pods were surface disinfested for 45 sec successively in 1.05% NaOCl, 70% ethanol and sterile water. Five hull pieces were plated on each of 5 plates of potato dextrose agar (PDA) and 10 plates of corn meal agar (CMA). Five of the CMA plates were incubated at 37° C for isolation of *P. myriotylum*. All other plates were incubated at 25° C. Identification of *P. myriotylum*, *R. solani* (AG4), *Fusarium* spp. and other fungi was based on standard references (Parmeter and Whitney, 1970; Van der Plaats-Niterink, 1981; Barnett and Hunter, 1987). Populations of *Pythium* spp. and *Fusarium* spp. in the soil were assessed by plating soil dilutions on selective media (Eckert and Tsao, 1962; Komada, 1975). Population of *R. solani* (Henis *et al.*, 1973) and *S. rolfsii* (Rodriguez-Kabana *et al.*, 1980) in soil were also determined.

Results and discussion

Of the 36 peanut fields surveyed in 1983 all but 16 were in pasture, grain sorghum, cotton or fallowed in 1984. In addition to 10 new locations, these 16 fields were resurveyed in 1984. In 1985, 15 of the peanut fields surveyed in 1984 and 1985 were in peanut and were sampled again. In addition, 31 new peanut fields were surveyed.

Pod rot, as diagnosed by symptoms and the presence of *P. myriotylum* or *R. solani* on pods was found in 41.7-73.1% of the peanut fields. Mean pod rot incidence for all fields in peanut was 6.1%, 21.3% and 5.8% for 1983, 1984 and 1985, respectively. The 21.3% incidence in 1984 may have been due to fewer fields in peanut and the high level of pod rot in some of the fields. Fifteen fields were in continuous peanut cultivation during the three year study. Generally, pod rot incidence in these fields increased from 1983 to 1984, but remained the same or declined in 1985 (Table I). Few of the individual fields showed a yearly progressive increase in pod rot incidence over the 3 years.

Pod rot was found on the cultivars Pronto, Spanco, Florunner, Starr and Comet, with the first three being the most commonly grown cultivars in Oklahoma. There were no significant differences (P = 0.05) in pod rot incidence between the cultivars.

Meloidogyne hapla Chitw., *Pratylenchus brachyurus* (Godfrey) Filipjev *et* S. Stekhoven, *Tylenchorhynchus* spp. and *Criconemella* spp. were the only plant-parasitic nematodes found in peanut fields (Table II). *Meloidogyne hapla* was frequently found in roots and soils (22.2-42.3% of the fields). In some fields, pods were severely galled (Fig. 1), indicating a highly virulent isolate of *M. hapla* producing galls on pods similar to those produced by *M. arenaria* (Unpublished data). The average annual population of *M. hapla* in infested fields ranged from 4-152.4 juveniles/100 cc soil and 45.9 to 220 juveniles/g root during the 3 year study.

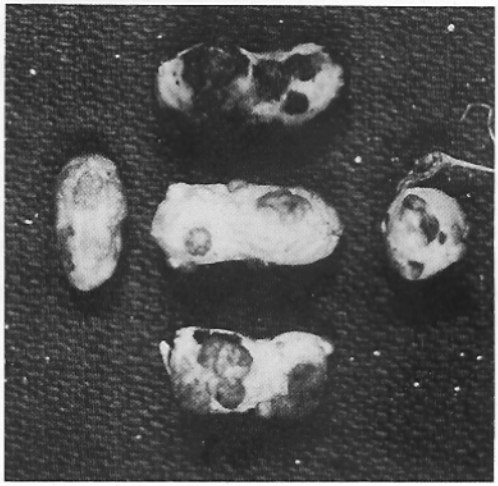

Fig. 1. Severely galled pods of 'Florunner' peanut infected by *Meiloidogyne hapla*.

Pratylenchus brachyurus was the second most common species encountered (Table II). Average yearly populations of *P. brachyurus* were 1-5.7 juveniles/100 cc soil and 8-21.5 juveniles/g root. *Tylenchorhynchus* spp. and *Criconemella* spp. were infrequently found in the peanut soils sampled (4- 240/100 cc soil and 12-92/100 cc soil, respectively).

Fungi commonly isolated from pods are given in Table II. *Pythium myriotylum* was found in 41.7-61.5% of the samples over 3 years. Rotted pods from three to 15 percent of the fields had only *P. myriotylum*. Other putatively identified *Pythium* spp. were *P. aphanidermatum* (Edson) Fitz. and *P. ultimum* Trow. Populations of *Pythium* spp. ranged from 0-836 propagules (p)/g soil. *Rhizoctonia solani* (AG4) was found in 30.4-34.6% of the fields, usually in combination with other fungi on rotted pods. Populations of *R. solani* were 0-47.4 p/100 g soil over the three year period. Twenty isolates of *R. solani* were identified as AG4 and 3 as AG2. Additionally, 12 isolates of binucleate Rhizoctonia-like-fungi were obtained from symptomatic pods.

Fusarium spp. were found in 34.8-41.7% of the fields. Most of these were identified as *F. solani* based on morphology of conidia, mycelial growth and the purple-pink colony color on Komada's medium (Komada, 1975). *Fusarium* populations were 0- 31,700 p/g soil. *Sclerotium rolfsii*

was isolated from rotted pods in 52.8-78.3% of the peanut fields over the 3 years. Populations of *S. rolfsii* were 0-34.0 p/500 g soil.

Species of *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Trichoderma*, *Macrophomina*, *Slerotinia* and *Botrytis* as well as many unidentified fungi were also isolated.

Our results suggest that the etiology of pod rot in Oklahoma ranges from simple to complex. *Pythium myriotylum* and *R. solani* (AG4) were frequently isolated from rotted pods. Their populations in soil were within the ranges reported to produce disease (Woodward and Jones, 1983; Filonow *et al.*, 1988). *Fusarium solani* which may interact with *P. myriotylum* in causing pod rot (Frank, 1972) was also commonly found. Our results suggest that *S. rolfsii* may be involved in pod rot in Oklahoma, possibly as a sole incitant. An individual fungal pathogen, e.g. *P. myriotylum*, appeared to be the sole incitant of pod rot symptoms in some fields, whereas a mixture of fungi were isolated from symptomatic pods in other fields.

Plant parasitic nematodes were frequently present in fields where a high incidence of pod rot was found (Table III). Of the fields infested with one or more species, 79-100% had pods exhibiting pod rot symptoms (Table III). *Meloidogyne hapla* was found in 86-100% of the fields with rotted pods harboring one or more of the fungi (Table II). *Meloidogyne hapla* was associated with *P. myriotylum* or *R. solani* (principal pod rot pathogens) in 43-82% of the fields. Average disease incidence in fields infested with plant parasitic nematodes was 15.1% (P = .05) compared with 7.8% for fields not containing these nematodes. Av-

TABLE I - *Pod rot incidence in Oklahoma fields continuously cropped to peanut over 3 years.*

Field	Incidence (%)		
	1983	1984	1985
B-15	15.0	11.7	0.0
B-21	0.0	25.0	20.0
C-6	0.0	15.0	0.0
C-9	0.0	33.0	18.7
C-10	14.2	13.3	17.8
C-11A	0.0	10.0	11.1
H-1A	13.7	33.3	33.3
H-1B	15.8	100.0	13.3
H2	2.0	6.7	0.0
H3A	0.0	0.0	5.0
H3B	0.0	6.7	8.3
H4	0.0	10.0	7.7
H6A	0.0	0.0	5.0
H6B	0.0	5.0	0.0
H7	6.7	15.0	15.0

TABLE II - *Frequency of occurrence of nematodes and fungi in Oklahoma peanut fields.*

Organism	Fields with nematodes and fungi (%)		
	1983	1984	1985
Nematodes			
Meloidogyne hapla	22.2	42.3	30.4
Pratylenchus brachyurus	22.2	7.7	4.3
Tylenchorynchus spp.	2.8	15.4	2.2
Criconemella spp.	8.3	0	0
Fungi			
Pythium myriotylum	41.7	61.5	43.5
Rhizoctonia solani (AG4)	30.5	34.6	30.4
Fusarium spp.	41.7	38.5	34.8
Sclerotium rolfsii	52.8	53.8	78.3
Other fungi	25.4	13.1	20.6

Percentage based on presence of fungus on pods and nematodes in roots or soil.

TABLE III - *Percent of nematode infested peanut fields with symptomatic pods or with symptomatic pods harboring fungi.*

Nematodes in field	Nematode infested fields (%)								
	With symptomatic pods [a]			With symptomatic pods harboring fungi listed in Table III			With symptomatic pods harboring *P. myriotylum* or *R. solani* (AG4)		
	1983	1984	1985	1983	1984	1985	1983	1984	1985
M. hapla	100	100	93	86	100	92	62	82	43
P. brachyurus	50	100	50	67	100	50	50	100	50
Criconemella spp.	100	0	0	100	0	0	33	0	0
Tylenchorhynchus spp.	0	100	100	0	100	100	0	100	100
two or more	79	100	88	93	100	93	56	86	53

[a] Discolored and/or decayed pods.

erage disease incidence in fields infested only with *M. hapla* was also greater (11.9%, P = .05) than in noninfested fields. In addition, mean populations of *P. myriotylum* or *S. rolfsii* in fields during the 3 years study were correlated (n = 16; r = 0.69 or r = 0.58, respectively) with populations of *M. hapla*. However, correlations with *M. hapla* populations and populations of *R. solani* or *Fusarium* spp. were r = 0.07 or r = 0.40, respectively.

Multiple regression analyses relating pod rot incidence (PI) to soil populations of *M. hapla* (Mh), *Pythium* spp. (P), *R. solani* (Rs), *S. rolfsii* (Sr) and *Fusarium* spp. (F) were also performed. Pod rot incidence in fields infested with *M. hapla* was best explained ($R^2 = 0.793$; P = .001; N = 18) by the model: % PI = 2.456 — 0.01 Mh + 0.041 P + 0.434 Rs + 2.956 (10^{-4}) F — 0.228 Sr.

Partitioning of sums of squares showed significant individual contributions only by *Pythium* spp., *M. hapla* and *R. solani* populations.

These results suggest that *M. hapla* and possibly other nematodes play an important role in pod rot etiology. *Meloidogyne arenaria* has been shown to enhance pod rot caused by *P. myriotylum* (Garcia and Mitchell, 1975). Invasion of peanut pods by *Aspergillus flavus* was not increased in the presence of *M. hapla* (Minton *et al.*, 1969), but Cylindrocladium black rot of peanut was enhanced in the presence of *M. hapla* or *Macroposthonia ornata* (Tayler) DeGrisse (= *C. ornata*), possibly due to the entry of the fungus in nematode-induced feeding wounds (Diomande and Beute, 1981). Studies are warranted to determine the nature and the mechanism(s) of interaction between *M. hapla* and *P. myriotylum* or *S. rolfsii*.

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