

Pythium Species Associated with Pod Rot on West Texas Peanuts and *In Vitro* Sensitivity of Isolates to Mefenoxam and Azoxystrobin¹

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ABSTRACT

A survey was conducted in 107 peanut fields to determine the association of *Rhizoctonia* or *Pythium* species in pod rot. *Rhizoctonia solani* and *Pythium* spp. were isolated from rotted pods in 35 and 39% of the fields, respectively. Isolates of *Pythium* were collected and 26 of the 85 isolates were identified to *Pythium* species, other than *P. oligandrum*. The three most common pathogenic species identified were *P. irregulare*, *P. myriotyllum*, and *P. ultimum*. The sensitivity of 47 isolates of *Pythium* to the fungicides mefenoxam and azoxystrobin was determined *in vitro*. Concentrations that reduced colony growth on agar by 50% (EC₅₀) ranged from 0.001–0.27 µl ai mefenoxam/ml, and from 1–103 µl ai azoxystrobin/ml.

Key Words: *Arachis hypogaea*, groundnut, *Pythium irregulare*, *Pythium myriotyllum*, *Pythium ultimum*.

Pod rot is the most consistent and frequent disease problem in the west Texas peanut producing area. Pod rot can be caused by a number of different fungi including *Rhizoctonia solani*, *Pythium* spp., *Sclerotium rolfsii*, and *Fusarium* spp. (Porter *et al.*, 1982). A survey of chemical usage on peanuts in the southwestern U.S. (Smith *et al.*, 1998) indicated that most of the fungicides used in Texas were active against *R. solani*, but not against *Pythium* spp. However, beginning in 1999, *Pythium* was isolated more frequently from pod rot samples sent to the Texas A&M University Agriculture and Extension Center - Lubbock than was *Rhizoctonia* (T. Wheeler, unpublished). There is, therefore, a need to clarify the relative frequency of both *Rhizoctonia* and *Pythium* in association with pod rot in this region.

Fungicides with activity against *Pythium* spp. and recommended for pod rot control or suppression include mefenoxam and azoxystrobin (Texas Peanut Production Guide, 2001). Mefenoxam is

a stable compound that is resistant to a wide range of pH, temperature, and light conditions (Sukul and Spiteller, 2000). When mefenoxam performs poorly it may be due to either biodegradation (Sukul and Spiteller, 2000) or fungal resistance (Davison and McKay, 1999). Insensitivity to metalaxyl by a *Pythium* species was first reported on turfgrass to *P. aphanidermatum* (Sanders, 1984). However, insensitivity to mefenoxam from other *Pythium* species has been reported only rarely (Moorman and Kim, 2004; Moorman *et al.*, 2002; Taylor *et al.*, 2002). In west Texas, mefenoxam has been used widely as a cotton seed treatment, but there has been relatively little use of mefenoxam in peanut production (Smith *et al.*, 1998). Biodegradation of mefenoxam is associated with extensive usage (Davison and McKay, 1999) and is probably not a factor.

Azoxystrobin has activity against a wide range of fungi, but oomycetes are generally less sensitive than the Ascomycetes, Dueteromycetes, or Basidiomycetes (Clough and Godfrey, 1998). Azoxystrobin is an effective and economical tool for managing mixtures of pathogens causing pod rots, particularly when both *Rhizoctonia* and *Pythium* spp. are involved. Fungal insensitivity to azoxystrobin has been documented in many fungi (Vinelli and Dixon, 2002; Wong and Wilcox, 2002; Mavroei and Shaw, 2005). An understanding of the sensitivity of *Pythium* spp. isolated from peanuts to azoxystrobin and mefenoxam is important for pod rot management in fields where pathogenic *Pythium* spp. are present.

Materials and Methods

Survey. Peanut fields (107) were selected without bias as to diseases in Cochran, Crosby, Dawson, Gaines, Hockley, Lubbock, Terry, and Yoakum counties in the Texas Southern High Plains. Fields were identified by driving through multiple (paved and dirt) roads in a county and marking the presence of peanut fields, and by flying over counties and marking the presence of peanut fields. Each field in a county received an identification number; then a series of random numbers were drawn and the subset of fields to be surveyed was selected. Each field was sampled in an identical manner regardless of size. Fields ranged from 12 to

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99 ha. Sampling began in the last week of August and continued throughout September of 2003. Fields sampled included Runner, Spanish, Virginia, and Valencia types. Varieties in some fields could be identified and included Flavorrunner 458 (most frequent), Florunner, NC7, Spanco, Tamsan 90, Valencia 101, VC2, and Viraguard. Maturity at the time of sampling ranged from only small, immature pods all the way to mature, ready for harvest pods.

Four transects were made across each field, with 10 locations sampled per transect. The sample location was selected by pacing even distances along the transect. At each sample location, approximately 1m of plants in a row were pulled up and all the discolored pods were collected. The soil underneath the plants was searched for any pods that had been dislodged from the plants. Pods with any discoloration in the 40 sample locations of a field were placed in a paper bag and refrigerated until the sample was processed. Processing (conducted within 1 wk of sampling) consisted of examining each pod for necrosis, weighing all necrotic pods, and selecting up to 16 pods for assay. In some cases, there were less than 16 pods from a field with necrosis. Pods selected for assays represented the range of symptoms expressed for a field (black, brown, white, rotted, and sunken type lesions) were washed, dried, and two small pieces (approximately 0.5 to 1 cm) were plated out on water agar and a *Pythium* selective medium (Lewis and Filonow, 1990). Plates were incubated at room temperature (21 to 23C) and monitored daily for mycelial growth, for seven days. Mycelia growing from these pod pieces were transferred to potato dextrose agar and identified to the genus level. Twenty-six of 85 isolates identified as belonging to the genus *Pythium* (no more than 4 isolates from a given field) were sub-cultured on corn meal agar (CMA) + 10 µg rifampicin/ml, and identified to species based on the descriptions from Plaats-Niterink (1981). There were 50 unidentified *Pythium* isolates that did not produce any structures that were useful for taxonomic purposes and nine identified as the nonpathogenic *P. oligandrum*. *Pythium* isolates that were selected for species identification represented all the fields where isolates were found, that did not quickly become contaminated by bacteria. The pathogens present within each field were divided into four classes: 1. neither *Rhizoctonia* nor *Pythium* present; 2. *Rhizoctonia* present without *Pythium*; 3. *Pythium* present without *Rhizoctonia*; or 4. *Rhizoctonia* and *Pythium* both present.

Fungicide Sensitivity. Isolates of *Pythium* spp. were maintained on CMA + 10 µg rifampicin/ml.

One cm² plugs of mycelium were placed on CMA plates containing the following concentrations of mefenoxam : 0, 0.0096, 0.096, 0.96, and 9.6 µl/ml of medium. The source of mefenoxam was Ridomil Gold EC (47.6% ai, Syngenta Crop Protection, Greensboro, NC). The size of the colony was measured approximately 40 hrs later. Percent inhibition was expressed as a percentage of the colony diameter in the absence of mefenoxam (Ridomil Gold EC). Plates were grown on a laboratory bench at room temperature which varied from 18–23 C. The plates were not kept in the dark, since mefenoxam is resistant to sunlight (Sukul and Spittler, 2000). Each isolate was tested on a given date with five concentrations of fungicide (including the 0 concentration) and three replications per isolate/concentration, arranged in a randomized complete block design. Each isolate was tested at least two times. Isolates were run in subgroups from November 03 through February 04.

Azoxystrobin was tested in a fashion similar to that of mefenoxam, except that higher concentrations were used (10 µl, 100 µl, 1,000 µl, and 4,000 µl/ml). The source of azoxystrobin was Abound FL (22.9% ai, Syngenta Crop Protection, Greensboro, NC).

The concentration that effectively reduced colony diameter by 50% (EC₅₀) was estimated for all isolates. A number of different models were tested to estimate EC₅₀. However, the natural log transformation of both fungicide concentration and percentage inhibition of fungal growth provided the most consistent fit for data.

Results and Discussion

Survey. Both *Pythium* and *Rhizoctonia* spp. were isolated from rotted pods in similar percentages. The classes representing *Rhizoctonia* alone, *Pythium* alone, and *Rhizoctonia* and *Pythium* together were found in 19, 23, and 16% of the fields surveyed, respectively. In 42% of the fields, neither *Rhizoctonia* nor *Pythium* were found. In those cases, either no pod rot was identified, or only superficial necrosis was present which is typical of the black hull fungus *Thielaviopsis basicola*. *Thielaviopsis basicola* was not isolated from the rotted pods, but would have required different selective media. *Sclerotium rolfii* was isolated from pods in 4% of the fields. *Fusarium* was isolated from pods in all fields. The overall condition of the pods was good, but when there was substantial rot, then typically either *Rhizoctonia* or *Pythium* were isolated. The 2003 summer was dry, particularly during the time of pod formation,

so the environment was not conducive for pod rot. All of the surveyed peanut fields were irrigated to some degree. The frequency of *Pythium* and *Rhizoctonia* spp. isolated from rotted pods in a survey can change from year to year (Hollowell *et al.*, 1998). In North Carolina, both *Pythium* spp. and *Rhizoctonia* spp. were the two pathogens most frequently isolated in a survey of peanut fields, but *Rhizoctonia* spp. predominated in one year, and *Pythium* spp. predominated in two years of a three year survey (Hollowell *et al.*, 1998). It is possible that the pod rot percentage in west Texas will increase in years where the environment is more conducive for disease, and that the relative percentages of disease due to *Pythium* and *Rhizoctonia* may change. However, it is clear that this region has pod rot associated with both *Rhizoctonia* spp. and *Pythium* spp., although pod rot overall during a dry summer is relatively low.

Pythium oligandrum was frequently isolated from rotted peanut pods, but is not considered a plant pathogen (Benhamou, *et al.*, 1999). Twenty-six *Pythium* isolates were identified to the species level, not including *P. oligandrum*. The species of *Pythium* most frequently isolated in west Texas were *P. ultimum* (9 isolates), *P. myriotylum* (10 isolates), and *P. irregulare* (6 isolates). One isolate was identified tentatively as *P. heterothallicum*. In a North Carolina survey, *P. irregulare* and *P. spinosum* were the species most frequently identified (Hollowell *et al.*, 1998). Other species identified in North Carolina on peanuts were *P. myriotylum*, *P. dissotocum*, *P. ultimum*, *P. vexans*, *P. dimorphum*, and *P. paroecandrum*. The “classic” *Pythium* species on peanut is considered to be *P. myriotylum* (Porter *et al.*, 1982).

Fungicide Sensitivity. The pathogenic *Pythium* isolates (those associated with pod rot, but not including *P. oligandrum*) had EC_{50} which ranged from 0.001–0.270 $\mu\text{l ai}$ mefenoxam/ml. Only one isolate was identified as *P. heterothallicum*, and it was quite sensitive to mefenoxam ($EC_{50} = 0.011 \mu\text{l ai}$ of mefenoxam/ml). Five isolates of *P. irregulare* that were obtained from three fields had EC_{50} that ranged from 0.049–0.173 $\mu\text{l ai}$ of mefenoxam/ml, with an average EC_{50} of 0.119 $\mu\text{l/ml}$. Seven isolates of *P. myriotylum* that were obtained from five fields, had EC_{50} that ranged from 0.012–0.163 $\mu\text{l ai}$ of mefenoxam/ml and averaged 0.074 $\mu\text{l/ml}$. Three isolates of *P. oligandrum* from three fields had EC_{50} that ranged from 0.008–0.099 $\mu\text{l ai}$ of mefenoxam/ml, and averaged 0.042 $\mu\text{l/ml}$. Two isolates of *P. ultimum* from two fields had EC_{50} of 0.005 and 0.006 $\mu\text{l ai}$ of mefenoxam/ml. Within the same field, isolates of *Pythium* could have very similar EC_{50} (G31A, B, and C ranged from 0.001 to

0.008 $\mu\text{l ai}$ of mefenoxam/ml), or very different EC_{50} (L3A and L3C were 0.033 and 0.270 $\mu\text{l ai}$ of mefenoxam/ml, respectively, Table 1). None of the isolates would be considered resistant to mefenoxam. Mefenoxam resistance has been defined as $EC_{50} > 100 \mu\text{l/ml}$ (Moorman *et al.*, 2002; Taylor *et al.*, 2002) and the least sensitive isolate (L3C) had an EC_{50} of 0.27 $\mu\text{l/ml}$. The EC_{50} of *P. ultimum* var. *sporangiiferum* from sugar beet ranged from 0.050–1.3 $\mu\text{l/ml}$ (Branter and Windels, 1998). The EC_{50} of *P. irregulare* obtained from apple roots was much higher (0.200–0.790 $\mu\text{l/ml}$) than from *P. ultimum* (0.010–0.170 $\mu\text{l/ml}$) (Mazzola *et al.*, 2002). *Pythium irregulare* that was obtained from plants taken from commercial greenhouses (a mixture of plant species) was frequently (21 of 57 isolates) resistant to mefenoxam, while no resistant *P. myriotylum*, and only 1 of 13 resistant *P. ultimum* isolates were found (Moorman *et al.*, 2002). The sensitivity of mefenoxam in the *Pythium* isolates examined could be attributed to their lack of exposure to this fungicide. Previous exposure was probably limited to cotton seed treatments, which would have placed minimal selection pressure on *Pythium* populations.

Sensitivity of *Pythium* spp. to azoxystrobin was much lower than to mefenoxam. However, some fungi use an alternative respiratory pathway that can interfere with the activity of azoxystrobin in a petri dish assay (Stevenson *et al.*, 2004). This means that these results cannot be compared directly to assays where salicylhydroxamic acid is used to inhibit *Pythium* from using an alternative respiratory pathway, though no published *in vitro* studies with azoxystrobin and *Pythium* were found. Sensitivity of different *Pythium* isolates to azoxystrobin in this study ranged from 1 to 103 $\mu\text{l ai}$ of azoxystrobin/ml. *Pythium heterothallicum* had an EC_{50} of 12 $\mu\text{l ai}$ of azoxystrobin/ml. The EC_{50} for *P. irregulare* ranged from 39–103 $\mu\text{l ai}$ of azoxystrobin/ml and averaged 62. The EC_{50} for *P. myriotylum* ranged from 5–25 $\mu\text{l ai}$ of azoxystrobin/ml and averaged 14.7. The EC_{50} for *P. oligandrum* ranged from 10–42 $\mu\text{l ai}$ of azoxystrobin/ml and averaged 23. The EC_{50} for *P. ultimum* was 14 for one isolate and no acceptable model could be fitted to the second isolate (Y6C). Isolate Y6C had a slight decrease in growth from 10 μl to 1,000 μl azoxystrobin/ml and no growth occurred at 4,000 μl azoxystrobin/ml. All the isolates of *P. irregulare* had less sensitivity to azoxystrobin than any of the other pathogenic *Pythium* isolates that were identified to species. Of the isolates that were not identified to species, the most sensitive *P. irregulare* isolate (G27A) was less sensitive than 93% of the remaining isolates.

Table 1. Mefenoxam and azoxystrobin concentrations that reduce growth of *Pythium* spp. by 50% (EC₅₀).

Isolate ^a	Species	EC _{50m} ^b	EC _{50a} ^c
Co1	<i>P. oligandrum</i>	0.008	10
Cr1A	<i>P. ultimum</i>	0.006	14
D22B	<i>Pythium</i> spp.	0.096	1
D22C	<i>Pythium</i> spp.	0.050	8
D24B	<i>P. myriotylum</i>	0.080	19
D3A	<i>Pythium</i> spp.	0.066	26
G2	<i>Pythium</i> spp.	0.007	13
G6B	<i>Pythium</i> spp.	0.005	19
G8A	<i>P. irregulare</i>	0.135	103
G8C	<i>P. irregulare</i>	0.049	61
G9B	<i>Pythium</i> spp.	0.010	5
G14A	<i>P. irregulare</i>	0.173	65
G15A	<i>Pythium</i> spp.	0.194	8
G15B	<i>Pythium</i> spp.	0.009	2
G15C	<i>Pythium</i> spp.	0.009	15
G18A	<i>Pythium</i> spp.	0.007	12
G18B	<i>Pythium</i> spp.	0.021	14
G19B	<i>P. myriotylum</i>	0.163	25
G26A	<i>P. myriotylum</i>	0.020	9
G26C	<i>Pythium</i> spp.	0.192	5
G27A	<i>P. irregulare</i>	0.132	39
G27C	<i>P. irregulare</i>	0.104	42
G30A	<i>Pythium</i> spp.	0.085	27
G30B	<i>Pythium</i> spp.	0.023	25
G30C	<i>Pythium</i> spp.	0.030	
G31A	<i>Pythium</i> spp.	0.008	
G31B	<i>Pythium</i> spp.	0.001	23
G31C	<i>Pythium</i> spp.	0.006	15
G32A	<i>Pythium</i> spp.	0.007	6
G32B	<i>P. oligandrum</i>	0.018	17
G32C	<i>P. heterothallicum</i>	0.011	12
G32D	<i>Pythium</i> spp.	0.013	9
GT14	<i>P. myriotylum</i>	0.012	14
GT19A	<i>Pythium</i> spp.	0.093	24
GT19B	<i>Pythium</i> spp.	0.040	13
H2	<i>P. oligandrum</i>	0.099	42
L3A	<i>Pythium</i> spp.	0.033	11
L3C	<i>Pythium</i> spp.	0.270	50
L6A	<i>Pythium</i> spp.	0.003	6
L6B	<i>Pythium</i> spp.	0.027	36
L6C	<i>Pythium</i> spp.	0.196	10
Y4A	<i>Pythium</i> spp.	0.005	7
Y6C	<i>P. ultimum</i>	0.005	
Y8A	<i>P. myriotylum</i>	0.115	15
Y8B	<i>P. myriotylum</i>	0.075	6
Y8C	<i>P. myriotylum</i>	0.051	15
Y9A	<i>Pythium</i> spp.	0.004	28

^aIsolates from the same field have the same first letter and number (example Y8), but differ in the last letter (example Y8A, Y8B, Y8C).

^bThe model used was $\log(\% \text{ Pythium growth}) = a + b \times \log(\text{concentration of mefenoxam in } \mu\text{l/ml})$ which was used to calculate the concentration of mefenoxam which reduced *Pythium* growth by 50% as EC_{50m}.

^cThe model used was $\log(\% \text{ Pythium growth}) = c + d \times \log(\text{concentration of azoxystrobin in } \mu\text{l/ml})$ which was used to calculate the concentration of azoxystrobin which reduced *Pythium* growth by 50% as EC_{50a}.

Summary and Conclusions

Pythium spp. are an important component of the pod rot complex in west Texas. Chemical management options for this organism include mefenoxam (control) and azoxystrobin (suppression). There do not appear to be resistance issues with respect to mefenoxam use on peanut. There is evidence in other crops and with certain fungal species, that resistance to azoxystrobin is associated with increased usage, and especially with low dosages of the product (Gisi *et al.*, 2000). The majority of azoxystrobin treatments are applied to control *Rhizoctonia* pod rot, and the recommended rate for this fungus is 37.4–74.9 g ai/ha of azoxystrobin. Typically, producers will use 50–56 g ai/ha for *Rhizoctonia* control (broadcast rate). This may be a sublethal rate to control *Pythium*, for which the required rate is 74.9 g ai/ha. Continued reliance on azoxystrobin at sublethal rates for *Pythium* spp. will probably lead to more isolates with reduced sensitivity to this fungicide.

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