Etiology of Peanut Pod Rot in Nicaragua: I. The Effect of Pod Size, Calcium, Fungicide, and Nematicide

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Abstract

Pod rot is one of the most important peanut (*Arachis hypogaea* L.) diseases in the pacific coast region of Cosiguina in Nicaragua. The region receives excessive rainfall during the peanut growing season and fields with high pod rot incidence often have moderate to high infestations of stem rot (*Sclerotium rolfsii*) and lesion nematode (*Pratylenchus* spp.). It was hypothesized that soilborne fungi, nematode damage and/or soil calcium deficiency could be involved in peanut pod rot. Flutolanil (1.2 kg a.i./ha, two applications), aldicarb (3.4 kg a.i./ha, one application), and calcium (670 kg/ha, two applications of gypsum) were evaluated on small (Georgia Green) and large (C-99R) seeded, runner cultivars in field experiments from 2005 to 2007 to help determine disease etiology. Experiments were split-split-plot or split-plot designs and treatments were replicated five times. There were no differences in pod rot between the two cultivars. Flutolanil did not decrease pod rot and only increased yield when associated with aldicarb nematicide. Gypsum application did not decrease pod rot, or increase yield or pod calcium content. Aldicarb had no effect on pod rot, but significantly increased yield by 22%, apparently by suppression of pod and/or root damage by lesion nematode. Neither calcium deficiency nor lesion nematode damage increased pod rot.

Introduction

Pod rot is one of the most important diseases affecting peanut production in Nicaragua. The disease is more prevalent in the pacific coast region of Cosiguina, as well as in Chinandega and Leon to a lesser extent. Peanut pod rot symptoms include brown to dark discoloration of the pericarp and pegs that usually begins at the apical pod tip and extends to the basal pod segment and peg. In addition to rotted pods, pegs often disintegrate and pods are lost in soil, further contributing to a yield reduction (7).

Preliminary observations in locations with high pod rot incidence in Nicaragua showed that lesion nematode populations were often high. Laboratory assays of pods with symptoms of lesion nematode damage (Fig. 1) indicated densities of 1456 to 2462 *Pratylenchus* spp. per 5 g of shells of Georgia Green. Lesion nematode injury can best be identified by the presence of small tan spots with dark centers on pods. The lesion nematode is common in the warmer climates of the world similar to that of Nicaragua, and can be found in large numbers inside the shells of mature peanut pods and pegs (15), where they colonize dark-colored, necrotic lesions (10). In a heavily infested field, Good et al. (10) found that peanut shells of a runner cultivar contained from six to eight times as many *P. brachyurus* nematodes as did the roots. The nematode feeds within the parenchymatous tissues, weakening the pegs and pods, and is recognized as an economically important pathogen of peanut in most production regions of the world (17). Although other nematode species have been implicated in the pod rot complex of peanut (8), no literature is available for pod rot caused by interactions between *Pratylenchus* spp. and

fungi. Nevertheless, Good et al. (10) found that in fields heavily infested by *P. brachyurus*, 19% of pods were left in the ground at digging because of weakened or rotted pegs associated with heavy infection by lesion nematode. Conversely, only 6% of the pods were left in soil in plots treated with nematicide. Since many fields with pod rot in Nicaragua also had high populations of lesion nematodes, it was important to determine the relationship between lesion nematode damage, peanut pod rot, and yield.

Fig. 1. Lesion nematode damage on peanut pods caused by *Pratylenchus* spp. in Cosiguina, Nicaragua.

Stem rot (*Sclerotium rolfsii*) is another important soilborne peanut disease in fields with pod rot prevalence in Nicaragua. *Sclerotium rolfsii* infects lower stems, roots, pegs, and pods during peanut pod formation and maturation, or earlier, and may cause pod rot (16) (Fig. 2). The association between *S. rolfsii* and other fungi in peanut pod rot has been reported as well (12), but not between *S. rolfsii* and *Pratylenchus* spp. nematodes. Flutolanil, a benzanilide fungicide, is registered for use on peanut and effective against *S. rolfsii* and *Rhizoctonia solani* (4), another fungal species isolated from pod rot samples from peanut fields in Nicaragua. Other isolated fungal species and their significance to peanut pod rot in these fields are discussed in a second manuscript of this series (2).

Fig. 2. Peanut pod rot caused by *Sclerotium rolfsii* in Cosiguina, Nicaragua. Note the uniform discoloration of infected pods, sclerotial initials of *S. rolfsii*, and the premature seed sprouting, which are typical of pod rot associated with *S. rolfsii*.

Another factor that has been shown to play a significant role in the peanut pod rot complex is low level of available calcium in soil (5), especially in large-seeded cultivars (9,11,14). Sumner et al. (18) indicated that pod surface area and surface to volume ratio were important in determining the quantity of calcium in seed and explains why large seeded varieties are more sensitive to calcium deficiency. Georgia Green, a small-seeded runner type, is the standard peanut cultivar in Nicaragua. However, other peanut cultivars with different pod and

seed sizes are being introduced to the country. Nearly all of the new cultivars have larger pods than Georgia Green (809 to 875 seeds/kg), with one of the largest being C-99R (605 to 678 seeds/kg). Due to its larger seed size, C-99R should be more susceptible than Georgia Green to pod rot induced by calcium deficiency. The relative susceptibility of these new cultivars to pod rot and comparing a large versus small-seeded cultivar would be an indicator of the importance of calcium nutrition to pod rot development in Nicaragua.

The objectives of the study were to understand the etiology of pod rot in Nicaragua, and to determine the effect of cultivar selection, fungicide, nematicide, and gypsum applications on pod rot and peanut pod yield.

Cultivar Selection and Fungicide, Nematicide, and Gypsum Treatments

Field experiments were conducted in seven locations in Cosiguina and one location in Leon regions in Nicaragua from 2005 to 2007 to evaluate the effect of cultivars (small-seeded Georgia Green and large-seeded C-99R), flutolanil, aldicarb, and gypsum applications on pod rot control and peanut pod yield. These inputs were chosen to selectively control factors known to cause pod rot, and identify those with the greatest impact in Nicaragua. *Sclerotium rolfsii* and *R. solani* were suspected to cause pod rot of peanut in Nicaragua, and flutolanil is one of the most effective fungicides used for disease control (4). Aldicarb is labeled for use on peanuts to control nematodes. It also has activity on thrips, but thrips damage has not been observed on peanuts in Nicaragua. Applications of gypsum were evaluated as a source of calcium, and were repeated to compensate for leaching by heavy rainfall.

Fields were selected based on past occurrences of pod rot and high populations of lesion nematodes. The fields were prepared by disk plowing 20 to 25 cm deep and disk harrowing three times before planting. The experimental design was either a split-split-plot or split-plot with treatments replicated five times. Each plot consisted of double beds (two twin rows per bed), the left bed for pod yield and the right bed for destructive sampling for nematode and pod rot assessments. Plots were 10 m long and separated by a 3-m fallow alley. Georgia Green and C-99R were planted with a twin-row Cole planter at 23 to 26 seeds/m. Row spacing was 109.2 cm between the outside rows and 73.7 cm between the inside rows.

In split-split-plot experiments, the whole-plot factor was fungicide, the sub-plot factor was nematicide, and the sub-sub-plot factor was gypsum. In the split-plot experiments, the whole-plot factor was cultivar and sub-plot factor was nematicide. Fungicide treatments were: flutolanil (1.2 kg a.i./ha); and nontreated control. Nematicide treatments were: aldicarb (3.4 kg a.i./ha); and nontreated control. Gypsum treatments were: at beginning pod (R3, 50 to 60 DAP) and beginning seed (R5, 80 to 90 DAP), as defined by Boote (3), with application of 670 kg/ha each time; and nontreated control.

The gypsum was applied by hand in a 50-cm band centered over the row without incorporation. The field sites had a medium level of extractable calcium with ammonium acetate extraction method (6) prior to planting (7.8, 7.6, and 7.4 meq Ca/100g soil in 2005, 2006, and 2007, respectively), but it was hypothesized that soil calcium deficiency might occur during pod formation and maturation by leaching of calcium during excessive rainfall or the calcium being in an unavailable form.

Flutolanil was applied at the same times as gypsum with a CO₂-belt-pack sprayer delivering 189 liter/ha at 31 psi by four 110 04 tips with 50 mesh screens per bed. Aldicarb was applied after planting, but before seedling emergence, with a Gandy applicator in a 30-cm band centered over the row and lightly incorporated with a rake. Standard production practices were applied uniformly to the field, including regular sprays with chlorothalonil (1.26 kg a.i./ha) for control of leaf spots and peanut rust. Plots were not fertilized or irrigated during the growing seasons.

Pod rot incidence was determined by counting the number of intact and rotted pods collected from plants in 1-m sections of each bed. Rotted pods left

in soil were manually excavated and included in counts. Since some pods would have been destroyed before harvest at full maturity, this evaluation was conducted approximately 100 DAP. Disease incidence was obtained by dividing the number of rotted pods by total number of pods in a section and multiplying by 100. Three 1-m sections were sampled in each plot, and the final disease incidence was an average of the three sections. In locations with high counts of lesion nematode, five 2.5-cm-diameter by 10-cm-deep soil cores were collected from the geocarposphere (1) of each plot within 2 weeks before peanut digging to determine nematode populations. In addition to soil samples, peanut pods (50 to 100 pods per plot) were collected during the same time period. Soil and pod samples were placed in plastic bags and transported in coolers to the LAQUISA Laboratorios Quimicos, S.A. (Carretera Leon, Managua km 83, Apartado 154, Leon, Nicaragua) for analysis of populations of plant-pathogenic nematodes. The nematode extraction procedure from soil was by the Baerman funnel method as described by Hooper (13). The extraction of nematodes from shells of pod samples was by the Baerman funnel method (10). Lesion nematodes were identified and counted using a stereo binocular microscope. A diagnostic key (15) was used for proper identification of nematode genus, but species were not determined. Only nematode counts of *Pratylenchus* spp. in shells are presented and discussed in this paper as counts of the other nematodes in shells and soil samples were inconclusive.

The left bed of each plot was mechanically dug and inverted with a KMC digger/inverter at about 130 to 150 DAP for yield assessment. Windrows were mechanically harvested with a two-row combine within 2 weeks. The final pod moisture content after air-drying was approximately 8% (wt/wt), and peanut yield was determined after removing foreign material. At digging, samples of 50 to 100 pods/plot were collected and sent to the LAQUISA laboratory for determining calcium content in shells and seed. Calcium content was analyzed by Mehlich No. 3 method previously described by Tucker (19).

The Statistical Analysis System PROC MIXED (SAS Institute Inc., Cary, NC) was used to analyze variation of nematode counts (especially *Pratylenchus* spp.), pod rot incidence, peanut pod yield, and calcium content in shells and seed in response to the treatments and cultivars. The interaction of year $\times$ location $\times$ treatments for nematode counts, pod rot, pod yield, and calcium content in shells and seed was used to determine if data could be pooled across years and locations. The Protected LSD ($\alpha = 0.05$) was used for mean separations among treatments.

Field experiments to evaluate the effect of nematicide and cultivars on pod rot and yield were conducted at two locations in Cosiguina in 2005 (Table 1). Neither cultivar nor aldicarb affected pod rot incidence, and both small-seeded Georgia Green and the large-seeded C-99R had relatively high pod rot. This experiment was not repeated in 2006 and 2007 because of the limited availability of seed for C-99R. In addition, grower cooperators had little interest in growing C-99R because it yielded significantly lower than Georgia Green.

Table 1. Effect of small-seeded (Georgia Green) and large-seeded (C-99R) cultivars and nematicide on pod rot and peanut pod yield in two fields at Cosiguina in 2005.

Cultivar	Nematicide	Pod rot incidence (%)	Peanut pod yield ^x (kg/ha)
Georgia Green	aldicarb	20.4	5766 a
	none	24.1	5611 a
C-99R	aldicarb	22.7	4701 b
	none	29.9	4689 b
LSD		ns	742

^x Means within a column with the same letter are not significantly different according to the LSD test ($\alpha \leq 0.05$).

ns: Indicates that means within a column are not significantly different according to ANOVA ($\alpha \leq 0.05$).

Multivariate analysis of variance across tests indicated that the number of *Pratylenchus* spp. nematodes in shells and pod rot incidence were both negatively correlated to pod yield. In contrast, calcium content in shells and total pod calcium were positively correlated to pod yield. However, the number of *Pratylenchus* spp. in shells and calcium content in shells and seed were not correlated to pod rot (Table 2).

Table 2. Partial correlation coefficients of pod calcium, *Pratylenchus* spp. count in shells, pod rot and pod yield in trials according to multivariate analysis of variance using Proc GLM SS3 of SAS.^x

DF = 28	Pod rot	Peanut pod yield
Calcium in shells	r = -0.164104 P = 0.3950	r = 0.258731 P = 0.0502
Calcium in seed	r = -0.051677 P = 0.7901	r = 0.194462 P = 0.3121
Total calcium (shells plus seed)	r = -0.020752 P = 0.9149	r = 0.247172 P = 0.0511
<i>Pratylenchus</i> spp. nematodes per 5 g of shells	r = 0.16914 P = 0.1337	r = -0.288370 P = 0.0376
Pod rot	1.000000	r = -0.501955 P = 0.0310

^x Partial correlation coefficients and probabilities between variables were from eight trials in Cosiguina and Leon regions from 2005 to 2007, r = partial correlation coefficient, P = probability value.

Application of aldicarb with or without flutolanil and calcium decreased ($P = 0.027$) the number of *Pratylenchus* spp. in shells. Aldicarb alone decreased the number of *Pratylenchus* spp. in shells by 73% compared to control plots. Application of aldicarb with or without flutolanil and calcium also increased ($P = 0.044$) pod yield compared to nontreated control plots (Table 3). However, aldicarb did not decrease pod rot and it had no effect on calcium content in shells and seed.

Table 3. Effect of fungicide, nematicide, and gypsum applications on pod (shell and seed) calcium, *Pratylenchus* spp. counts, pod rot and pod yield from five trials in Cosiguina and one trial in La Leona (Leon), 2005 to 2007.^w

Fungicide	Nematicide	Gypsum	Calcium content ^x (%)			Number of <i>Pratylenchus</i> spp. per 5 g of shells ^y	Pod rot (%)	Peanut pod yield ^z (kg/ha)
			shell	seed	total			
flutolanil	aldicarb	yes	0.24	0.07	0.31	599 b	24.0	5691 a
		no	0.19	0.07	0.26	700 b	24.9	5414 ab
	none	yes	0.22	0.08	0.30	2292 a	27.2	4888 bc
		no	0.20	0.07	0.27	2031 a	27.5	4859 bc
none	aldicarb	yes	0.22	0.07	0.29	535 b	25.2	5306 ab
		no	0.19	0.07	0.26	655 b	25.9	5156 ab
	none	yes	0.22	0.08	0.30	2377 a	29.8	4824 bc
		no	0.19	0.06	0.25	2462 a	32.0	4409 c
LSD			ns	ns	ns	998	ns	744

^w Georgia Green cultivar was planted at 23 to 28 seeds/m.

^x Pod samples were collected at digging. Total calcium content refers to calcium in shells plus seed.

^y Samples were collected 2 weeks prior to digging.

^z Means in columns with the same letter(s) are not significantly different according to the protected LSD ($\alpha \leq 0.05$).

ns: Indicates that means within a column are not significantly different according to ANOVA ($\alpha \leq 0.05$).

Flutolanil increased pod yield only when accompanied by aldicarb irrespective of gypsum application. Flutolanil did not decrease pod rot and it had no effect on pod calcium content and nematode counts (Table 3).

Gypsum applications at beginning pod (R3) and beginning seed (R5) had no effect on pod rot, nematode counts, or pod yield. Applications of gypsum generally made little difference in pod calcium content (Table 3).

Conclusions

While stem rot is an important disease in these fields and *S. rolfii* can be a very effective pod rot pathogen, the results indicated that it played only a minor role in peanut pod rot in these trials. The results also indicated that infection and damage by *Pratylenchus* spp. can be significant and justifies further research, but apparently the damage is not associated with pod rot symptoms. The lack of a differential response of pod rot to gypsum in the small-seeded or large-seeded cultivar provided evidence that calcium deficiency was not a primary cause of pod rot in these fields. However, there was a positive correlation between pod yield and calcium content in shells and total pod calcium. This may have been due to smaller, positive effects on peanut seed quality or grade that were not of large enough magnitude to impact pod rot.

Overall, this study indicated that factors other than calcium deficiency, nematode damage, or *S. rolfii* are causing the majority of pod rot. Another common cause of pod rot is infection by *Pythium* spp., and data presented in the second paper of this series indicates that this pathogen was a primary cause of pod rot in Nicaragua (2).

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