Journal of Nematology 36(2):167–170. 2004.

Relationship between *Meloidogyne arenaria* and Aflatoxin Contamination in Peanut

P. Timper,[1] D. M. Wilson,[2] C. C. Holbrook,[1] and B. W. Maw[3]

Abstract: Damaged and developing kernels of peanut (*Arachis hypogaea*) are susceptible to colonization by fungi in the *Aspergillus flavus* group which, under certain conditions, produces aflatoxins prior to harvest. Our objective was to determine whether infection of peanut roots and pods by *Meloidogyne arenaria* increases aflatoxin contamination of the kernels when peanut is subjected to drought stress. The experiment was a completely randomized 2-x-2 factorial with 6 replicates/treatment. The treatment factors were nematodes (plus and minus *M. arenaria*) and fungus (plus and minus *A. flavus* inoculum). The experiment was conducted in 2001 and 2002 in microplots under an automatic rain-out shelter. In treatments where *A. flavus* inoculum was added, aflatoxin concentrations were high (> 1,000 ppb) and not affected by nematode infection; in treatments without added fungal inoculum, aflatoxin concentrations were greater ($P \leq 0.05$) in kernels from nematode-infected plants (1,190 ppb) than in kernels from uninfected plants (79 ppb). There was also an increase in aflatoxin contamination of kernels with increasing pod galling ($r^2 = 0.83$ in 2001, $r^2 = 0.43$ in 2002; $P \leq 0.04$). Colonization of kernels by *A. flavus* increased with increasing pod galling ($r^2 = 0.18$; $P = 0.04$) in 2001 but not in 2002. Root-knot nematodes may have a greater role in enhancing aflatoxin contamination of peanut when conditions are not optimal for growth and aflatoxin production by fungi in the *A. flavus* group.

Key words: aflatoxin, *Arachis hypogaea, Aspergillus flavus, A. parasiticus,* interaction, *Meloidogyne arenaria,* peanut, root-knot nematode.

Damaged and developing seed kernels of peanut (*Arachis hypogaea*) are susceptible to colonization by *Aspergillus flavus* and *A. parasiticus* prior to harvest (Cole et al., 1995; Sanders et al., 1985). These two closely related species are often referred to as the *A. flavus* group. After colonization of the kernel, these fungi can produce toxic metabolites known as aflatoxins under certain conditions. Aflatoxins are a major concern for the peanut industry because they are potent carcinogens. In the United States, visible signs of fungal growth on peanut kernels result in segregation of farmer lots from the edible category and a loss of $2.6 million per year to peanut producers (Lamb and Sternitzke, 2001).

The conditions that favor aflatoxin contamination of peanuts include a combination of drought stress and high soil temperatures 3 to 6 weeks prior to optimum peanut maturity (Blankenship et al., 1984; Hill et al., 1983; Sanders et al., 1985; Wilson and Stansell, 1983). Damaged and immature pods are more susceptible to infection by the *A. flavus* group than are sound, mature kernels (Hill et al., 1983; Sanders et al., 1985). Peanut pods damaged by the lesser cornstalk borer (*Elasmopalpus lignosellus*), even when the damage was superficial, had increased invasion by the *A. flavus* group and subsequent aflatoxin production in drought-stressed plants (Bowen and Mack, 1993; Lynch and Wilson, 1991; Lynch et al., 1990). Likewise, nematode damage to pods may increase aflatoxin contamination of peanut kernels under drought conditions.

Meloidogyne arenaria, race 1, infests 25% of the peanut acreage in the southeastern United States (Bridges et al., 1994). In peanut, the nematode infects the roots, pegs, and developing pods. Galls produced on the pod are generally superficial; however, this damage may create conditions favorable for invasion of *A. flavus* and *A. parasiticus*. Although Minton and Jackson (1967) found no interaction between *M. arenaria* and either *A. flavus* colonization or aflatoxin contamination of peanut kernels, their experiments were performed before it was understood that aflatoxin production is favored by drought stress and high temperatures. Therefore, our objective was to determine whether infection of peanut by *M. arenaria* increases aflatoxin contamination of the kernels when peanut is subjected to drought stress.

Materials and Methods

The experiment was a completely randomized 2-x-2 factorial with 6 replicates/treatment. The treatment factors were nematodes (plus and minus *M. arenaria* inoculum) and fungus (plus and minus *A. flavus* group inoculum). The experiment was conducted in 2001 and 2002 in microplots under an automatic rain-out shelter located in Tifton, Georgia. Each of the 24 microplots was 1.7 × 1.4 m and was surrounded by pre-cast concrete barriers 8 cm wide and 91 cm deep. The rain-out shelters were equipped with a fiberglass cover that closed automatically whenever rain fell. The soil within the plots was a sand (92% sand, 5% silt, 3% clay, and 0.38% carbon) that had been fumigated in the spring of each year with methyl bromide at 483 kg/ha to reduce densities of plant-parasitic nematodes.

Peanut seeds (cultivar Georgia Green) were planted into 72-cell (38-cm^3) polystyrene trays (Speedling Inc., Sun City, FL) containing a 50:50 mix of loamy sand and potting mix (70 to 80% fine peat moss, perlite, dolomitic limestone, and gypsum). Two to 3 weeks after planting, a squirt bottle was used to inoculate each tray

Received for publication 9 October 2003.
[1] USDA ARS, P.O. Box 748, Tifton, GA 31793.
[2] Department of Plant Pathology, University of Georgia, P.O. Box 748, Tifton, GA 31793.
[3] Department of Biological and Agricultural Engineering, University of Georgia, P.O. Box 748, Tifton, GA 31793.
The authors thank William Wilson, Bonnie Evans, David Clements, and Mamadou Coulibaly for their technical assistance on this project and D. Rowland and K. Bowen for reviewing a draft of this manuscript.
E-mail: ptimper@tifton.usda.gov
This paper was edited by J. L. Starr.

with 576,000 (2001) and 360,000 (2002) eggs of *M. arenaria* that had been extracted from 'Rutgers' tomato with NaOCl (Hussey and Barker, 1973). The eggs, in a 150-ml suspension, were applied evenly over the tray resulting in approximately 8,000 and 5,000 eggs/plant in 2001 and 2002, respectively. The peanut seedlings, with and without inoculum of *M. arenaria,* were transplanted into microplots 1 week after inoculation. Transplanting was done on 31 May 2001 and on 17 May 2002. The peanut seedlings were planted 10/row in two rows 91 cm apart and 1.4 m long. During plant growth and pod set, the plots were left uncovered during rainfall and irrigated when plants showed the first symptoms of wilt. Fungicide and insecticides were applied according to recommendations for peanut in Georgia (Guillebeau, 2002). Nematode-treated plots received additional nematode inoculum 67 to 68 days after transplanting to increase the likelihood of pod infection. This was done by using a 500-ml squirt bottle to apply 250,000 eggs evenly over the plant canopy of each row (ca. 25,000 eggs/plant). All plots were irrigated immediately afterward with 2.5 cm of water to wash the eggs off foliage.

Inoculum of the *A. flavus* group was prepared using the method of Holbrook et al. (1994). Briefly, roasted cracked corn was inoculated with spore suspensions of *A. flavus* NRRL 3357 and *A. parasiticus* NRRL 2999 and incubated at 25 °C for 3 days. The inoculum was then stored at −20 °C for no more than 2 months until needed. After the plants had started to bloom, half the plots were inoculated with the *A. flavus* group by sprinkling 56 g of the infested corn over the plant canopy of each row. This was done 42 and 61 days after transplanting in 2001 and 2002, respectively. Drought was induced 12 weeks after transplanting by ceasing supplemental irrigation and setting the shelters to automatically cover the plots during rainfall.

The peanuts were dug and inverted 17 and 19 weeks after transplanting in 2001 and 2002, respectively. Pod- and root-gall indices were determined for all 20 plants and an average calculated for each plot. The 1-to-5 index was based on an estimate of the percentage of the root system or pods from each plant with galls (1 = 0 galls, 2 = 1% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%). Pods from each plot were bulked and air-dried to 7% moisture. The percentage colonization of both shells and kernels by the *A. flavus* group was determined by first surface sterilizing pods (n = 46 to 100/plot) in 0.5% NaOCl for 5 minutes and aseptically removing the kernel. The sterilized shells and kernels were then plated on 2% malt extract agar amended with 10% NaCl. The remaining pods were shelled, and the kernels pooled and ground. Typically, a 100-g sample of ground kernels was used to determine aflatoxin concentration by the immunoaffinity column fluorometer method (Trucksess et al., 1991). However, in a few plots, peanut yields were small and a sample of <100 g but >30 g was used. The fluorometer was calibrated from 0 to 400 ppb aflatoxin. Samples exceeding 400 ppb were diluted 10-fold and then reanalyzed. A second 10-fold dilution was done if the samples exceeded 4,000 ppb. The maximum detection level for aflatoxins in this study was 40,000 ppb.

Analysis of variance was used to determine the effect of three factors (nematodes, fungal inoculum, and year) on aflatoxin concentration, percentage invasion of the shell, and colonization of the kernel by the *A. flavus* group. Prior to analysis, aflatoxin concentration was transformed by Ln (ppb + 1) and percentages were transformed by arcsine ($\sqrt{p}$ + 1). Because the presence of a few kernels with extremely high concentrations of aflatoxin can lead to large aflatoxin values for the entire sample (Cucullu et al., 1966), the highest aflatoxin concentration was discarded for each treatment per year, thus leaving five replications per treatment. The relationship between pod galling and either aflatoxin concentration (Ln-transformed), invasion of the shell, or colonization of the kernel was determined by regression analysis. These regressions were also determined for root galling.

Results

At the end of the growing season, most of the peanut plants were severely wilted and some were dead. In plants inoculated with *M. arenaria,* root galling was similar in 2001 and 2002, with an average index of 4.4 ± 0.09 (±SE). Pod galling, on the other hand, was greater (P < 0.0001) in 2001 than in 2002, with average indices of 3.4 ± 0.16 and 2.0 ± 0.08, respectively. In plants not inoculated with *M. arenaria,* there was a small amount of root (1.08) and pod galling (1.08) in 2001 but not in 2002.

Aflatoxin concentrations in kernels were greater in 2002 than in 2001 (1,994 vs. 256 ppb; $P \leq 0.0001$). Because there was no interaction between year and any of the other factors, the data for the two years were combined. Neither the presence of *M. arenaria* nor inoculation with the *A. flavus* group affected aflatoxin production; however, there was an interaction between these factors (P = 0.0008). In treatments where *A. flavus* inoculum was added, aflatoxin concentrations were high but not affected by nematode infection; whereas in treatments without added fungal inoculum, aflatoxin concentrations were greater in kernels from nematode-infected plants than in kernels from uninfected plants (Fig. 1). In plots without fungal inoculum, there was an increase ($P \leq 0.04$) in aflatoxin contamination of kernels with increasing root and pod galling (Fig. 2, data for root galling not shown). Pod galling accounted for 83% of the variation in aflatoxin concentration in 2001; however, the strength of the relationship with aflatoxin was much less for pod galling in 2002 and for root galling in both years (r^2 between 0.43 and 0.57).

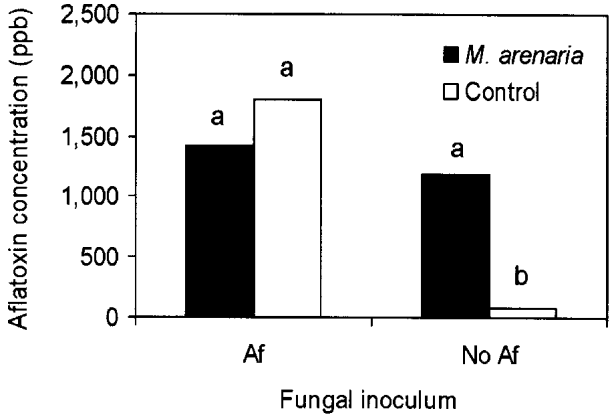

FIG. 1. Aflatoxin concentration in peanut kernels from plots with and without inoculum of the *Aspergillus flavus* group (Af), and with and without inoculum of *Meloidogyne arenaria*. Bars within a grouping with the same letter are not different ($P > 0.05$).

The percentage of kernels and shells colonized by the *A. flavus* group was not affected by nematodes, fungal inoculum, or the interaction of these two factors. However, there was an effect of year on colonization, with a greater ($P < 0.0001$) percentage of kernels and shells colonized by the fungi in 2002 than in 2001 (36 vs. 5% of kernels and 69 vs. 29% of shells). Colonization of kernels by *A. flavus* increased with increasing pod galling ($P = 0.04$, $r^2 = 0.18$) in 2001 but not in 2002.

Discussion

The results of this study indicate that infection of peanut by *M. arenaria* can lead to an increase in aflatoxin contamination of peanut kernels when the plants are subjected to drought stress during pod maturation. The study was conducted under drought stress to create conditions conducive to aflatoxin contamination (Blankenship et al., 1984; Hill et al., 1983; Sanders et al., 1985; Wilson and Stansell, 1983). It is not known whether *M. arenaria* infection increases aflatoxin contamination of peanut when adequate soil moisture is available.

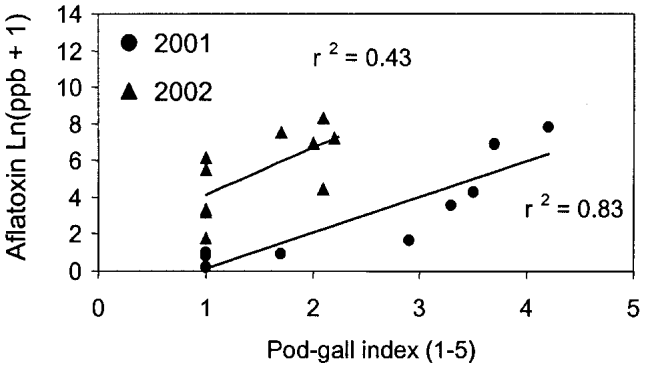

FIG. 2. Relationship between pod galling from *Meloidogyne arenaria* and aflatoxin concentrations in peanut kernels. Data are from plots without inoculum of the *Aspergillus flavus* group. The equation for 2001 is Y = 1.9X − 1.8 ($P = 0.0003$) and for 2002 is Y = 2.6X + 1.6 ($P = 0.04$). The gall index was based on the percentage of pods with galls: 1 = 0, 2 = 1% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%. Pod index was determined from 20 plants/plot, whereas aflatoxin concentration was determined from a 100-g sample of ground kernels per plot.

Nematode infection increased aflatoxin contamination of kernels only in plots without *A. flavus/A. parasiticus* inoculum. The *A. flavus* group is ubiquitous in nature (Bowen and Mack, 1993); therefore, it is not surprising that these fungi were present in plots without fungal inoculum. In plots receiving fungal inoculum, high populations of the *A. flavus* group and subsequent aflatoxin production may have masked the enhancing effects of the nematode. The high aflatoxin concentrations in 2002 also may have partially masked the effects of the nematode. In 2001, there was a strong relationship between pod galling and aflatoxin concentration, whereas in 2002, this relationship was much weaker. Mean daily air temperatures were 3 °C warmer in 2002 than in 2001 during the later half of the growing season. These warmer conditions probably contributed to the greater aflatoxin concentration. Root-knot nematodes may have a greater role in enhancing aflatoxin contamination of peanut when conditions are not optimal for growth and aflatoxin production by *Aspergillus* spp.

Both root and pod galling from *M. arenaria* were correlated with aflatoxin concentration; however, the two indices were also positively correlated with each other. Aflatoxin concentration and colonization of the kernels by the *A. flavus* group appeared to be more closely associated with pod than with root galling. In 2001, there was abundant pod galling and a strong relationship between pod galling and aflatoxin concentrations; whereas, root galling, which was abundant in both years of the study, had a weaker relationship with aflatoxin concentrations. We did not consistently find a relationship between *A. flavus* colonization of kernels and either root or pod galling; only in 2001 was pod galling positively correlated with the percentage of kernels colonized by the fungi. In a similar study conducted in greenhouse pots, the presence of *M. arenaria* did not increase the percentage of peanut kernels colonized by the *A. flavus* group (Minton and Jackson, 1967). However, in a microplot study, Minton et al. (1969) found a greater incidence and density of *A. flavus* in peanut kernels from plots infested with *Meloidogyne hapla* than from plots infested with only the fungus, though aflatoxin was not detected in kernels from any of the treatments. The inconsistent effect of nematode infection on colonization of kernels by the *A. flavus* group may be due to other factors that affect the susceptibility of the kernels to fungal infection such as kernel maturity and insect damage. In the current study, a greater percentage of kernels were colonized by the *A. flavus* group in 2002 than in 2001. Perhaps, the enhancing effect of the nematodes on colonization of kernels by toxigenic *Aspergillus* spp. is only apparent when other risk factors for colonization are low.

The mechanism by which nematodes increase aflatoxin contamination of peanut is unknown. It is possible that nematode infection leads to greater colonization of the kernel by fungi in the *A. flavus* group, and when the plants are subjected to heat and water stress, aflatoxin production is enhanced. Galls on the peanut pods may increase kernel colonization by *Aspergillus* spp. by serving as entry points for the fungi or, in cases of severe galling, by delaying or preventing kernel development (Minton, 1984). Small and immature kernels have a higher incidence of *A. flavus* and are thought to be more susceptible to colonization than mature kernels (Sanders et al., 1985). Root galling may also contribute to aflatoxin production. Nematode damage impairs root function, which can predispose plants to drought stress. However, Sanders et al. (1993) showed that drought stress around the pods contributed to preharvest aflatoxin production whereas drought stress in the root zone did not. Nematode infection of roots also may cause physiological changes in the plant that increase the susceptibility of kernels to infection by toxigenic *Aspergillus* spp. Indeed, nematodes are known to increase plant suscepbility to fungal disease (Abawi and Chen, 1998). In some cases, the effect of the nematode is systemic, resulting in increased fungal infection or disease severity when the fungus and nematode are physically separated (Faulkner et al., 1970; Sidhu and Webster, 1977).

It is important to understand how *M. arenaria* increases preharvest aflatoxin levels in peanut. If nematodes are increasing aflatoxin in heavily galled, immature pods, then the resulting small kernels could be mechanically segregated from the sound, mature kernels. However, if nematodes are increasing aflatoxin levels in mature peanuts with no or superficial pod galling, then an effective management strategy for these nematodes is critical for reducing aflatoxin contamination in non-irrigated peanut fields. The most effective strategy for managing *M. arenaria* may be nematode-resistant peanut cultivars because they suppress nematode reproduction throughout the growing season. Nematicides and crop rotation result in lower at-planting densities of *M. arenaria,* but populations may increase during the growing season resulting in yield loss and a greater risk of preharvest aflatoxin contamination in non-irrigated peanut.

Literature Cited

Abawi, G. S., and J. Chen. 1998. Concomitant pathogen and pest interactions. Pp. 135–158 *in* K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant and nematode interactions. Madison, WI: American Society of Agronomy.

Blankenship, P. D., R. J. Cole, T. H. Sanders, and R. A. Hill. 1984. Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85:69–74.

Bowen, K. L., and T. P. Mack. 1993. Relationship of damage from the lesser cornstalk borer to *Aspergillus flavus* contamination in peanuts. Journal of Entomological Science 28:29–42.

Bridges, D. C., C. K. Kvien, J. E. Hook, and C. R. J. Stark. 1994. Nematodes for the peanut crop in the Southeast market ranked by yield loss caused by pests considered independently and value of crop loss by pests. Appendix 1.4 P. 1 *in* An analysis of the use and benefits of pesticides in U.S.-grown peanuts: I Southeastern production region. Tifton, GA: National Environmentally Sound Production Agriculture Laboratory.

Cole, R. J., J. W. Dorner, and C. C. Holbrook. 1995. Advances in mycotoxin elimination and resistance. Pp. 456–474 *in* H. E. Pattee and H. T. Stalker, eds. Advances in peanut science. Stillwater, OK: American Peanut Research and Education Society.

Cucullu, A. F., L. S. Lee, R. Y. Mayne, and L. A. Goldblatt. 1966. Determination of aflatoxins in individual peanuts and peanut sections. Journal of the American Oil Chemist's Society 43:89–92.

Faulkner, L. R., W. J. Bolander, and C. B. Skotland. 1970. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint: Influence of the nematode as determined by a double root technique. Phytopathology 60:100–103.

Guillebeau, P., ed. 2002. Georgia pest control handbook: Commercial edition, Special Bulletin 28. Athens, GA: Cooperative Extension Service, University of Georgia.

Hill, R. A., P. D. Blankenship, R. J. Cole, and T. H. Sanders. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Applied and Environmental Microbiology 45:628–633.

Holbrook, C. C., M. E. Matheron, D. M. Wilson, W. F. Anderson, M. E. Will, and A. J. Norden. 1994. Development of a large-scale field system for screening peanut for resistance to preharvest aflatoxin contamination. Peanut Science 21:20–22.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

Lamb, M. C., and D. A. Sternitzke. 2001. Cost of aflatoxin to the farmer, buying point, and sheller segments of the southeast United States peanut industry. Peanut Science 28:59–63.

Lynch, R. E., and D. M. Wilson. 1991. Enhanced infection of peanut, *Arachis hypogaea* L., seeds with *Aspergillus flavus* group fungi due to external scarification of peanut pods by lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Peanut Science 18:110–116.

Lynch, R. E., D. M. Wilson, and B. W. Maw. 1990. Enhanced aflatoxin contamination of peanut as a result of insect damage to pods. Proceedings of the American Peanut Research and Education Society 22:78 (Abstr.).

Minton, N. A. 1984. Nematode parasites of peanuts. Pp. 373–394 *in* W. R. Nickle, ed. Plant and insect nematodes. New York: Marcel Dekker.

Minton, N. A., D. K. Bell, and B. Doupnik, Jr. 1969. Peanut pod invasion by *Aspergillus flavus* in the presence of *Meloidogyne hapla.* Journal of Nematology 4:318–320.

Minton, N. A., and C. R. Jackson. 1967. Invasion of peanut pods by *Aspergillus flavus* and other fungi in the presence of root-knot nematodes. Oleagineux 22:543–546.

Sanders, T. H., R. J. Cole, P. D. Blankenship, and R. A. Hill. 1985. Relation of environmental stress duration to *Aspergillus flavus* invasion and aflatoxin production in preharvest peanuts. Peanut Science 12:90–93.

Sanders, T. H., R. J. Cole, P. D. Blankenship, and J. W. Dorner. 1993. Aflatoxin contamination of peanuts from plants drought stressed in pod and root zone. Peanut Science 20:5–8.

Sidhu, G. S., and J. M. Webster. 1977. Predisposition of tomato to the wilt fungus (*Fusarium oxysporum lycopersici*) by the root-knot nematode (*Meloidogyne incognita*). Nematologica 23:436–442.

Trucksess, M. W., M. E. Stack, S. Nesheim, S. W. Page, R. H. Albert, T. J. Hansen, and K. F. Donahue. 1991. Immunoaffinity column coupled with solution fluorometry or liquid-chromatography postcolumn derivatization for determination of aflatoxins in corn, peanuts, and peanut butter: Collaborative study. Journal of the Association of Official Analytical Chemists 74:81–88.

Wilson, D. M., and J. R. Stansell. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Science 10:54–56.