A Survey of Peanut Disease, Nematodes and Insects
and Their Relationship to Pre-Harvest Aflatoxin Contamination Risk

A Final Report to the Georgia Commodity Commission for Peanuts

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Introduction

Multiple studies were carried out in 2010 and in 2011 to assess the relationship of the peanut root-knot nematode (*Meloidogyne arenaria*) and the ring nematode (*Criconemoides ornata*) on contamination of peanut with aflatoxin. It is understood that stress and damage, both of which can be caused by these plant parasitic nematodes, can increase the susceptibility of the peanut plant to infection by the fungus *Aspergillus flavus*. Aflatoxin is a metabolite produced by *A. flavus*. In accordance with research being conducted at Auburn University by Dr. Kira Bowen, the primary objective of this research was to determine if the level of aflatoxin present in peanuts at harvest could be correlated to populations of the peanut root-knot and ring nematodes during the season. If such a relationship could be established in Georgia, then it might be possible to advance the concept of a risk index (much like the UGA Tomato Spotted Wilt Risk Index and Peanut Rx) for improved management of aflatoxin in peanut production.

Materials and Methods

Initially in 2010, technicians were trained to use the VICAM system for the detection of aflatoxin peanut (see protocol in next section) and assessed peanut from and used this training to assess a peanut study from Tifton, GA for aflatoxin (see Table 1). Surveys of commercial peanut fields were carried out in 2010 to determine the relationship between populations of nematodes and the contamination of peanuts with aflatoxin. During the season, soil samples were collected form commercial peanut fields and analyzed for populations of the peanut root-knot and the ring nematodes (nematodes/100cc soil). At harvest, random peanut samples were collected from as many of these fields as possible and analyzed for aflatoxin using the same protocol as mentioned above (Table 2). In addition, peanut samples were collected from commercial peanut loads that were declared "Seg 2" because of damage from the burrower bug to determine if such resulted in an increase of aflatoxin (Table 3).

In 2011, additional surveys and field trials were carried out to determine the relationship between plant parasitic nematodes of peanut and aflatoxin. In addition to collecting soil samples for nematode analysis, plant samples in some instances were assessed for damage form the root-knot nematodes and tillage practices were considered. Peanut samples from each harvested plot were analyzed as described for aflatoxin (Table 4 and Table 5).

Results and Discussion

Aflatoxin levels were generally low in commercial peanut fields in 2010 and the levels did not appear to be correlated to populations of plant parasitic nematodes or to damage to burrower bugs. From studies in 2011, again, the levels of aflatoxin were generally below 20 ppb and did not appear closely tied to the populations of nematodes. The level of aflatoxin did appear to be closely tied to sampling where damaged seeds tended to have higher levels of aflatoxin as would be expected.

Acknowledgements

I would like to express my appreciation to the Georgia Peanut Commission and to the peanut farmers in Georgia for their support of these studies.

PEANUT AFLATOXIN STUDY: a protocol

Material and Methods for the analysis of aflatoxin in peanut using the VICAM methodology (as used to train technicians who conducted the analysis in this study).

Materials:

- Ground peanuts
- NaCl
- 80/20 Methanol /DI water
- Afla B columns
- Developer (5ml Bromine: 45ml of HPCL water)
- HPCL water
- Methanol

Method:

Flourometer Calibration

- Insert red vial and set at 240 ppb, press enter.
- Insert green vial and press enter.
- Insert yellow vial. The reading should be 120 ppb
- Take HPCL water samples from containers and make sure it's not contaminated, reading should be 0.

Sample Preparation

1. Place peanuts in a sack to be dried in an oven, should be set 10 degrees above outside temperature.
2. Shell and clean peanuts.
3. Grind peanuts

Sample Extraction

4. Weigh 100 grams of ground peanuts and place in a blender.

*If less than 100 grams, use 1g/2ml solvent; if less than 10 grams, use 20ml solvent.

5. Add 10 grams of NaCl.
6. Add 200 ml of 80/20 Methanol/DI water.
7. Cover blender and blend at high speed for 1 minute.
8. Pour about 50ml extract into fluted filter paper; collect filtrate in a clean container.

Extraction Dilution

9. Pipet 5ml filtered extract into test tube.
10. Dilute extract with 20ml of HPCL water and mix well.

11. Filter diluted extract through glass microfiber filter.

Aflatest Affinity Chromatography Procedure

12. Rinse syringe with HPCL water.
13. Place column on syringe and add filtered extract:

Method I	10ml for 0-40 ppb	0-40	divide by 10
Method II	1ml for 0-400 ppb	0-400	results are correct
Method III	100µL for 0-4,000 ppb		multiply by 10
Method IV	10µL for 0-40,000 ppb		multiply by 100

14. Pass extract half-way through column.
15. Wash column with 10ml HPCL water, stopping at the top of the packing.
16. Repeat the column wash with an equal volume of HPCL water. Pass all water completely through the column.
17. Collect the aflatoxin in a cuvette by passing 1ml of HPCL grade Methanol through the column.
18. Add 1ml of Aflatest Developer, mix well, and place cuvette in flourometer.

Table 1. 2010 AflaGuard/Aflatoxin Trial		
TRT#	**TRT NAME**	**AFLATOXIN RESULT**
1	Tilt Bravo Abound Bravo WS	**226.00**
2	Tilt Bravo Abound Bravo WS **Aflaguard**	**11.70**
3	Tilt Bravo Abound Bravo WS **Aflaguard**	**1024.12**
4	Tilt Bravo Provost Bravo WS **Aflaguard**	**1124.8**
5	Tilt Bravo Provost Bravo WS **Aflaguard**	**5.48**

Table 2. Survey (2010) of commercial fields to assess the relationship between plant parasitic nematodes and aflatoxin in peanut.

FARM	COUNTY	ROOT KNOT (per 100cc soil)	RING (per 100cc soil)	AFLATOXIN (ppb)
Rick LaGuardia	Miller	150	160	4.6
Rick LaGuardia	Miller	40	298	4.6
Rick LaGuardia	Miller	20	86	4.6
Andy Spooner	Miller	134	82	2.7
Andy Spooner	Miller	60	54	2.7
Andy Spooner	Miller	6	30	2.7
LN Cleveland	Miller	322	68	8.1
LN Cleveland	Miller	534	32	8.1
LN Cleveland	Miller	352	32	8.1
Mark Hanna	Seminole	8	70	3.4
Mark Hanna	Seminole	6	22	3.4
Mark Hanna	Seminole	8	186	3.4
Brad Thompson	Seminole	4	132	6.3
Brad Thompson	Seminole	0	24	6.3
Brad Thompson	Seminole	0	32	6.3
Newberry	Calhoun	216	36	4.5
Newberry	Calhoun	380	40	4.5
Newberry	Calhoun	180	48	4.5
Webb	Calhoun	2	72	1.5
Webb	Calhoun	0	268	1.5
Webb	Calhoun	0	66	1.5
Tim James	Ben Hill	36	7	2.9
Tim James	Ben Hill	234	14	2.9
Tim James	Ben Hill	42	12	2.9
Chip Dorminy	Ben Hill	6	7	12
Chip Dorminy	Ben Hill	45	20	12
Chip Dorminy	Ben Hill	36	26	12
Halin Reeves	Ben Hill	14	100	2.1
Halin Reeves	Ben Hill	110	44	2.1
Halin Reeves	Ben Hill	156	132	2.1
Stanley Harper	Irwin	0	12	2.5
Stanley Harper	Irwin	0	19	2.5
Stanley Harper	Irwin	0	17	2.5
Lamar Purvis	Irwin	0	284	4
Lamar Purvis	Irwin	0	546	4
Lamar Purvis	Irwin	0	26	4
Ted Milliron	Randolph	44	67	5
Ted Milliron	Randolph	104	22	5
Ted Milliron	Randolph	69	122	5
Billy Moore	Randolph	4	6	3
Billy Moore	Randolph	19	2	3
Billy Moore	Randolph	0	4	3

Table 2, continued.

FARM	COUNTY	ROOT KNOT (per 100cc soil)	RING (per 100cc soil)	AFLATOXIN (ppb)
Brad Carter	Randolph	0	6	4
Brad Carter	Randolph	0	1	4
Brad Carter	Randolph	0	1	4
Shivers 1	Clay	3	13	6.9
Shivers 1	Clay	73	4	6.9
Shivers 1	Clay	218	4	6.9
Shivers 2	Clay	70	14	2.9
Shivers 2	Clay	68	46	2.9
Shivers 2	Clay	122	42	2.9
Catherall	Clay	3	90	4.9
Catherall	Clay	14	9	4.9
Catherall	Clay	8	13	4.9
Sudderth	Calhoun	0	174	
Sudderth	Calhoun	8	102	
Sudderth	Calhoun	0	156	
Roach	Mitchell	20	46	
Roach	Mitchell	40	38	
Roach	Mitchell	100	20	
Roach	Mitchell	30	44	
Roach	Mitchell	142	82	
Roach	Mitchell	110	108	
Lanier	Berrien	0	40	
Lanier	Berrien	0	970	
Watson	Berrien	4	170	
Watson	Berrien	0	294	
Watson	Berrien	0	220	
Wilson	Turner	0	4	
Wilson	Turner	1	8	
Wilson	Turner	0	55	
Driggers	Turner	0	37	
Driggers	Turner	1	4	
Driggers	Turner	1	12	
Ross	Irwin	0	61	
Ross	Irwin	0	150	
Ross	Irwin	0	310	
Sinkbeil 1	Worth	32	12	
Sinkbeil 1	Worth	2	58	
Sinkbeil 1	Worth	0	38	
Sinkbeil 2	East Worth	0	122	
Sinkbeil 2	East Worth	0	104	
Benson Dryland	Tift	6	930	
Benson Dryland	Tift	0	504	
Benson Dryland	Tift	0	340	

Table 2 continued.				
FARM	COUNTY	ROOT KNOT (per 100cc soil)	RING (per 100cc soil)	AFLATOXIN (ppb)
Gerbel	Early	0	6	
Gerbel	Early	0	0	
Gerbel	Early	0	1	
Crapy	Early	8	70	
Crapy	Early	6	22	
Crapy	Early	8	186	
Turner Farm		0	0	
Turner Farm		0	0	
Hood Farm		0	1	
Hood Farm		0	1	
Mercer Farm		0	60	
Mercer Farm		0	9	
Johnson Farm		0	8	
Johnson Farm		0	1	
Knox Farm #6704 David Adams				2.1
Knox Farm #2611 David Adams	Colquitt			1.7
Bruce Farm #786 David Adams	Colquitt			4
Moultrie GA BTR Farms	Colquitt			3.7
Barney, GA Jeffrey L.& Lynn Tillman	Colquitt			1.2

Table 3. 2010 Analysis of peanut seed from various commercial fields classified as "Seg 2" due to burrower bug damage.

BAG DESCRIPTION	**AFLATOXIN (ppb)**
Trailer #68	**2.3**
Trailer # 56	**2.6**
Danforth 80	**2.6**
Stevens	**4.2**
Danforth 124	**2.1**
Brooks Co. Ex 6 Johnny Hogan Peanuts	**3.9**
Danforth 116	**3**
Danforth 20	**2.3**
Danforth H1C5	**4.1**
Chop Joiner Farm#2846 Trailer BCT 230 95#U366273	**2.5**
Danforth 53	**4.3**
Danforth 44	**5.4**
E & A Joiner Farm#2232 Trailer #BCT 266	**3.2**
E & A Joiner Farm # 2232 Trailer # CPC90	**2.9**
No Tag	**3.7**
Bruce Farms - 1	**10**
Bruce Farms D54	**5.3**
Knox Farms - 1	**3**
Bruce Farms D257	**9**
Bruce Farms - 2	**5**
Knox Farms - 2	**8**
Thomas Griffin DEM 68	**3.2**
Bruce Farms - 3	**3**
Knox Farms - 3	**5**
Bruce Farms - 4	**2.9**
Knox Farms D-65	**14**
Knox Farms - 4	**6**
Knox Farms D82	**5**
Bruce Farms D53	**13**
Bruce Farms - 5	**10**
Bruce Farms DEM 81	**3.9**
Knox Farms DEM 78	**3.4**
Knox Farms DEM 52	**6.5**

TREATMENT LEGEND for **Table 4** (below). Peanut Nematicide/Aflatoxin Trial									
1	2	3	4	5	6	7	8	9	10
GA06	**GA06**	**GA06**	**GA06**	**GA06**	**TIFGUARD**	**TIFGUARD**	**TIFGUARD**	**TIFGUARD**	**TIFGUARD**
Telone II THIMET	**Telone II THIMET TEMIK**	**Nemout Transporter THIMET Nemout Transporter**	**TEMIK**	**THIMET**	**Telone II THIMET**	**Telone II THIMET TEMIK**	**Nemout Transporter THIMET Nemout Transporter**	**TEMIK**	**THIMET**

Table 4. Peanut nematicide/aflatoxin trial; Blackshank Farm, Tifton, GA.

TRT #	DESCRIPTION		AFLATOXIN (ppb)	8-Jun		28-Oct	
				Root Knot (per 100cc soil)	Ring (per 100cc soil)	Root Knot (per 100cc soil)	Ring (per 100cc soil)
1	101	R	15	4	17	4	176
1	101	W	8.2	4	17	4	176
1	202	R	7.4	64	7	12	62
1	202	W	11	64	7	12	62
1	305	R	12	129	12	430	25
1	305	W	21	129	12	430	25
1	404	R	15	3	9	633	38
1	404	W	30	3	9	633	38
1	507	R	8.4	19	2	4	152
1	507	W	25	19	2	4	152
1	601	R	16	9	7	0	245
1	601	W	17	9	7	0	245
2	102	R	6.4	3	24	1	92
2	102	W	12	3	24	1	92
2	201	R	13	7	6	142	38
2	201	W	17	7	6	142	38
2	308	R	19	30	6	1	22
2	308	W	36	30	6	1	22
2	406	R	12	4	14	86	36
2	406	W	23	4	14	86	36
2	508	R	14	5	0	1	12
2	508	W	12	5	0	1	12
2	605	R	140	3	3	3	43
2	605	W	22	3	3	3	43
3	103	R	9.9	5	14	1198	62
3	103	W	12	5	14	1198	62
3	210	R	8.4	187	7	2	43
3	210	W	89	187	7	2	43
3	309	R	11	118	3	4	35
3	309	W	200	118	3	4	35
3	403	R	17	49	0	1	32
3	403	W	53	49	0	1	32

Table 4 continued. Peanut nematicide/aflatoxin trial; Blackshank Farm, Tifton, GA.							
3	501	R	33	27	12	650	41
3	501	W	54	27	12	650	41
3	610	R	37	20	16	934	86
3	610	W	64	20	16	934	86
4	104	R	7.1	1	13	136	64
4	104	W	17	1	13	136	64
4	206	R	15	112	13	772	28
4	206	W	45	112	13	772	28
4	304	R	18	437	10	730	113
4	304	W	45	437	10	730	113
4	409	R	17	38	6	536	94
4	409	W	38	38	6	536	94
4	504	R	19	0	6	341	125
4	504	W	61	0	6	341	125
4	606	R	26	6	1	473	167
4	606	W	43	6	1	473	167
5	105	R	12	3	17	426	22
5	105	W	9.5	3	17	426	22
5	208	R	8.4	121	4	0	35
5	208	W	38	121	4	0	35
5	301	R	2.6	26	3	231	6
5	301	W	4.7	26	3	231	6
5	405	R	15	5	0	391	51
5	405	W	31	5	0	391	51
5	509	R	14	3	1	63	10
5	509	W	50	3	1	63	10
5	603	R	11	1	6	280	28
5	603	W	13	1	6	280	28
6	106	R	8.6	3	12	0	80
6	106	W	12	3	12	0	80
6	207	R	33	125	6	16	49
6	207	W	13	125	6	16	49
6	303	R	23	284	17	14	39
6	303	W	80	284	17	14	39
6	402	R	15	24	4	42	31
6	402	W	39	24	4	42	31
6	503	R	15	38	7	2	417
6	503	W	32	38	7	2	417
6	602	R	14	0	5	0	249
6	602	W	22	0	5	0	249
7	107	R	11	7	23	0	29
7	107	W	34	7	23	0	29
7	209	R	8.9	25	5	380	69
7	209	W	25	25	5	380	69

Table 4 continued. Peanut nematicide/aflatoxin trial; Blackshank Farm, Tifton, GA.							
7	306	R	12	35	8	2	49
7	306	W	19	35	8	2	49
7	410	R	19	50	5	6	254
7	410	W	48	50	5	6	254
7	502	R	18	4	15	24	149
7	502	W	73	4	15	24	149
7	604	R	18	20	6	0	383
7	604	W	40	20	6	0	383
8	108	R	6.5	10	33	50	110
8	108	W	15	10	33	50	110
8	205	R	11	45	15	344	48
8	205	W	14	45	15	344	48
8	310	R	12	75	21	129	270
8	310	W	28	75	21	129	270
8	401	R	9.6	89	9	206	129
8	401	W	18	89	9	206	129
8	506	R	51	15	6	39	77
8	506	W	21	15	6	39	77
8	609	R	25	19	7	111	41
8	609	W	28	19	7	111	41
9	109	R	21	68	23	48	58
9	109	W	15	68	23	48	58
9	203	R	11	34	12	72	16
9	203	W	12	34	12	72	16
9	307	R	21	3	2	14	8
9	307	W	22	3	2	14	8
9	408	R	39	13	14	0	61
9	408	W	31	13	14	0	61
9	510	R	16	5	11	242	47
9	510	W	78	5	11	242	47
9	607	R	13	1	11	332	59
9	607	W	20	1	11	332	59
10	110	R	6.7	11	10	178	48
10	110	W	13	11	10	178	48
10	204	R	37	14	11	172	34
10	204	W	15	14	11	172	34
10	302	R	6.7	309	14	6	167
10	302	W	25	309	14	6	167
10	407	R	14	24	16	0	222
10	407	W	34	24	16	0	222
10	505	R	30	2	5	73	335
10	505	W	24	2	5	73	335
10	608	R	38	15	106	488	100
10	608	W	100	15	106	488	100

Table 5. 2011 analysis of peanut tillage study for relationship between damage from the peanut root-knot nematode and aflatoxin, Stripling Irrigation Park.

DESCRIPTION	Aflatoxin (ppb)	% ROOT GALL (ave 10 plants)	Tillage Strip=1 Conven=2	IRRIGATION X=0 Others=1	ROOT KNOT NEMATODE (100 cc soil)
203-235 R-10	2.4	11.2	1	0	
203-235 W-10	8				
209-299 R-16	1.3	43.3	1	1	
209-299 W-16	4.5				
104-143 R-6	4.7	33.5	1	0	
104-143 W-6	2.7				
201-218 R-12	3	3.9	2	1	
201-218W-12	4.9				
204-242 R-13	2.1	2	2	1	0
204-242 W-13	2.8				
108-182 R-8	2.1	1.4	2	1	
108-182 W-8	2.4				
101-115 R-1	2.7	47.6	1	1	
101-115 W-1	2.6				
406-464 R-33	0.86	4	2	1	26
406-464 W-33	1.6				
402-421 R-29	2.4	14.7	1	1	
402-421 W-29	3				
309-393 R-27	1.3	2.8	1	1	
309-393 W-27	3.3				
307-375 R-25	1.8	47.5	1	1	
307-375 W-25	3.7				
405-458 R-32	2	2.1	2	1	
405-458 W-32	2.6				
407-475 R-36	2.5	25.7	1	0	
407-475 W-36	100				
302-328 R-20	2.3	3	2	1	48
302-328 W-20	3.7				
305-354 R-23	3.1	2.5	2	0	
305-354 W-23	5.7				
301-312 R-19	3.2	6.4	2	1	
301-312 W-19	3.7				
202-224 R-11	2.8	5.8	2	1	
202-224 W-11	4				
208-283 R-17	1.4	15.9	1	1	66
208-283 W-17	2.1				

Table 5. Continued, 2011 analysis of peanut tillage study for relationship between damage from the peanut root-knot nematode and aflatoxin, Stripling Irrigation Park.

DESCRIPTION	Aflatoxin (ppb)	% ROOT GALL (ave 10 plants)	Tillage Strip=1 Conven=2	IRRIGATION X=0 Others=1	ROOT KNOT NEMATODE (100 cc soil)
103-137 R-3	3.9	7.1	1	1	
103-137 W-3	7.3				
306-366 R-22	1.1	4	2	1	
306-366 W-22	4				
102-129 R-2	1.7	30.3	1	1	228
102-129 W-2	2.5				
207-277 R-18	1.4	17.3	1	1	
207-277 W-18	3.5				
105-158 R-5	1.1	4.5	2	0	
105-158 W-5	1.7				
404-443 R-31	1.4	16.8	1	1	
404-443 W-31	2				
109-194 R-9	2.9	7.7	2	1	
109-194 W-9	2				
308-381 R-26	2.3	19.8	1	1	
308-381 W-26	2.4				
403-437 R-28	2.3	14.7	1	1	
403-437 W-28	20				
409-496 R-34	170	1.3	2	0	
409-496 W-34	1300				
401-419 R-30	16	20.9	1	1	20
401-419 W-30	2.3				
303-337 R-21	1.6	14.6	1	1	510
303-337 W-21	4				
206-266 R-15	1.9	1.1	2	0	2
206-266 W-15	29				
408-482 R-35	2.9	3.7	2	1	
408-482 W-35	2.2				
304-349 R-24	1.5	16.3	1	0	48
304-349 W-24	3.5				
107-176 R-7	8.5	1.3	2	1	5
107-176 W-7	3.6				
205-251 R-14	4.5	12.9	1	1	
205-251 W-14	20				