Development of a Large-Scale Field System for Screening Peanut for Resistance to Preharvest Aflatoxin Contamination[1]

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ABSTRACT

Preharvest aflatoxin contamination (PAC) of peanut occurs under prolonged periods of drought and heat stress. Evaluation of peanut germplasm may identify valuable sources of resistance to PAC, but will require a large scale screening system. The objective of this research was to develop a large-scale field system for screening peanut germplasm for resistance to PAC at Yuma, Arizona. Yuma is located in a desert and has great potential as a site for evaluating germplasm for resistance to PAC. Field studies were conducted in 1990 to determine if aflatoxin contamination would occur in drought stressed peanuts grown at Yuma. Aflatoxin levels up to 2,260 ppb were observed, however, 52% of the plots escaped contamination and the coefficient of variation (C.V.) were unacceptably large. During testing at Yuma in 1990 it was noted that drought stressed plants died quickly due to the rapid exhaustion of soil moisture. A subsurface irrigation system was installed in 1991 to alleviate this problem and allow for an extended period of drought stress in the pod zone. Results for 1991 showed a greatly increased mean aflatoxin contamination, a 50% reduction in the C.V., and a virtual elimination in the occurrence of escapes. A study was conducted in 1992 to compare plots with and without subsurface irrigation to determine if the differences observed between 1990 and 1991 were due to the use of subsurface irrigation. The use of subsurface irrigation in 1992 increased the mean contamination by over 100%, reduced the C.V. by over 50%, and reduced the percentage of escapes by over 90%.

Key Words: Aflatoxin, *Arachis hypogaea*, *Aspergillus flavus*, *Aspergillus parasiticus*, germplasm evaluation, peanut.

Progress in breeding peanut with resistance to preharvest aflatoxin contamination (PAC) has been extremely limited. Most breeding efforts to reduce aflatoxin contamination in peanuts have utilized a screen which measures *Aspergillus flavus* sporulation on rehydrated sound mature kernels that have been inoculated and incubated *in vitro* (Mixon and Rogers, 1973; Mixon, 1986; LaPrade *et al.*, 1973; Mehan *et al.*, 1981; Zambettakis *et al.*, 1981). Progress in developing cultivars with resistance to aflatoxin contamination has been minimized by the poor correlation between this laboratory screening technique and aflatoxin contamination under field (Blankenship *et al.*, 1985) or storage (Wilson *et al.*, 1977) conditions.

Breeding progress should be enhanced by the development of a screening technique that evaluates preharvest aflatoxin contamination under field conditions. It is well established that a period of at least 20 days of heat and drought stress immediately before harvest may result in aflatoxin contamination (Cole *et al.*, 1985; Sanders *et al.*, 1985). The difficulty has been in developing a system which can be used in a large-scale breeding effort. The objective of this study was to develop a field screening system which would be applicable to a large amount of peanut germplasm.

Materials and Methods

Field experiments were conducted at the Yuma Mesa Agricultural Experiment Station on superstition sand (81-7-12 sand-silt-clay). All experiments were conducted using either Florunner or Pronto, two cultivars which are susceptible to PAC. Two-row plots (1.5 x 1.8 m) were seeded at 4 seeds per 30 cm. *Aspergillus* inoculum was prepared using corn as an organic carrier. A light green conidial suspension (10 ml per four ounces of corn of *A. flavus* Link ex Fries (NRRL 3357) or *A. parasiticus* Speare (NRRL 2999) was grown for three days at room temperature (24-27 C) on sterile moisture equilibrated cracked corn (25% moisture). Plots were inoculated approximately 60 d after planting and each plot received 57 g of *A. flavus* (NRRL 3357) and 57 g of *A. parasiticus* inoculum.

Seeds were planted 14 May, 1990 and plots were irrigated as needed using flood irrigation. Shade cloth was erected over 36 plots on 5 September to examine the effect of reduced soil temperature on PAC. The last irrigation was applied 24 August. Plots were then subjected to a natural drought stress and harvested 24 September.

A delayed planting was used to determine if a fall drought stress would result in greater PAC than a summer drought stress at Yuma. Seeds were planted 20 June, 1990 and plots were irrigated as needed using flood irrigation.The last irrigation was applied 1 October. Plots were then subjected to a natural drought stress and harvested 15 November.

In 1991, seeds were planted 3 June and plots were irrigated as needed using flood irrigation through 23 August. Irrigations after this date were applied using subsurface irrigation tubing. Soil moisture was monitored using gypsum blocks buried 5, 15, 30 and 46 cm deep at four locations in the field. Soil temperature was monitored at a depth of 5 cm using a Ryan Tempmentor (Ryan Instruments, Redmond, WA). Temperatures were recorded each hour from a single sensor in each of the three treatments (i.e. bare soil, plant canopy, and shade and plant canopy). Pods were harvested 28 October.

In 1992, seeds were planted 11 May and plots were irrigated as needed using flood irrigation through 10 August. Forty-nine plots did not receive any irrigation after this date and 96 plots received subsurface irrigation. Pods were harvested 14 October.

Harvested pods were dried to 7% moisture and hand sorted to remove visibly damaged pods. Peanuts were shelled using a Peerless peanut sheller and ground in a household food processor for about one minute. Aflatoxin contamination was measured on a 100 g subsample with the immunoaffinity column fluorometer method (Trucksees *et al.*, 1991). The fluorometer was calibrated from 0 to 400 ppb. If the initial sample analysis indicated contamination above 400 ppb, then a 1 to 10 dilution of the extract was made and the sample was reanalyzed. If the reanalyzed sample indicated contamination above 4,000 ppb, then an additional 1 to 10 dilution and analysis were performed. The maximum contamination detectable using this method is 40,000 ppb.

Results and Discussion

An extended period of hot and dry conditions is needed during pod fill to ensure aflatoxin contamination in peanut genotypes susceptible to PAC. Yuma, Arizona is a desert

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environment with an average yearly rainfall of 7.5 cm. Most of the rain comes from late autumn through early spring, with sporadic low amounts occurring during the summer. No rainfall occurred at Yuma from 1 July 1990 through 31 October 1990 (Table 1). Cole *et al.* (1985) suggested that the upper mean soil temperature limit for development of aflatoxin contamination in undamaged peanuts was between 29.6 - 31.3 C. Average 5 cm bare soil temperatures at Yuma during July - September can be above this limit (Table 1). A summer drought stress under shade cloth and a fall drought stress period were evaluated in the event that soil temperature in the summer drought stress without shade was above the limit for aflatoxin contamination. The use of shade cloth reduced the maximum and average soil temperature during the summer stress period (Table 2). The drought-stressed plant canopy alone also reduced average soil temperature.

Table 1. Air and soil temperatures and rainfall at Yuma, Arizona during the peanut pod filling period in 1990.

	Air temperature (C)			Soil temperature†	
Month	Min	Max	Average	5 cm average (C)	Rain (cm)
July	26	41	33	34	0
August	24	39	32	33	0
September	22	38	29	32	0
October	15	33	23	24	0

† Bare soil temperature.

Table 2. Effect of peanut canopy and shade cloth on soil temperature (5 cm depth) at Yuma, Arizona in 1990.

	Soil temperature† (C)		
Treatment	Min	Max	Average
Plant canopy and shade	21	28	25
Plant canopy no shade	21	33	28
Bare soil	21	47	33

† Soil temperatures from the period 5 September to 24 September.

Table 3. Preharvest aflatoxin contamination of drought stressed peanut grown at Yuma, Arizona in 1990 using three screening systems.

			Aflatoxin contamination			
Stress period	Shade cloth	No. of samples	Mean (ppb)	C.V. (%)	Escapes[1] Number	%
Summer	No	65	228	223	28	43
Summer	Yes	36	140	463	26	72
Fall	No	36	0	—	36	100

[1]Escape = sample with < 5 ppb.

The use of subsurface irrigation in 1991 improved the potential for this site to serve as a resistance screening location. The use of this system greatly increased the mean aflatoxin contamination, reduced the C.V. by about 50%, and virtually eliminated the occurrence of escapes (Table 5). Ninety-seven percent of the plots in Yuma were contaminated with aflatoxin (mean contamination = 1,785 ppb) in 1991. This was a dramatic improvement over 1990, when no subsurface irrigation was used and pods in 52% of the plots

In 1990, aflatoxin contamination levels up to 2,260 ppb were observed in unshaded plots, however, the number of escapes and the coefficient of variation (C.V.) were unacceptably large (Table 3). Peanuts from shaded plots had less aflatoxin contamination and a larger percentage of escapes. No aflatoxin contamination was observed in peanuts from plots subjected to a fall stress. This was probably due to soil temperatures below the lower limit for aflatoxin contamination. Cole *et al.* (1985) observed a lower limit of between 25.7 - 26.3 C.

During testing at Yuma in 1990, drought-stressed plants died quickly due to the rapid exhaustion of soil moisture. A subsurface irrigation system was installed in 1991 to alleviate this problem and allow for an extended period of drought stress in the pod zone. Mean gypsum block readings (Table 4) showed that the subsurface irrigation system was effective in imposing an extended period of drought stress conditions in the pod zone (5 cm depth). Sanders *et al.* (1993) demonstrated that drought stress in the pod zone as opposed to the root zone is necessary and adequate for aflatoxin contamination to occur. We tried to provide only enough subsurface irrigation to maintain the life of drought stressed plants (Table 4).

Table 4. Mean gypsum block readings† from four soil depths using subsurface irrigation at Yuma, Arizona in 1991.

Soil depth	August		September											October	
(cm)	** 27	29	01	* 05	* 07	10	* 16	19	23	* 27	30	03	* 07		
5	84	63	26	6	5	2	1	0	0	0	0	0	0		
15	92	88	74	31	24	16	8	3	1	1	1	0	1		
30	93	91	80	53	62	38	37	15	4	20	7	4	30		
46	94	93	90	75	76	60	56	34	11	35	25	19	54		

†Gypsum block readings range from 100 (≥ -.25 kPa) to 0 (< -200 kPa).
** 8/23 - Last furrow irrigation.
* 9/2, 9/6, 9/11, 9/25 and 10/4 - subsurface irrigations.

Table 5. Preharvest aflatoxin contamination of drought stressed peanut grown at Yuma, Arizona in 1991 using subsurface irrigation.

		Aflatoxin contamination			
	No. of	Mean	C.V.	Escapes[1]	
Genotype	samples	(ppb)	(%)	Number	%
Florunner	96	1,167	102	4	4
Pronto	87	2,467	181	2	2

[1]Escape = sample with < 5 ppb.

Table 6. Preharvest aflatoxin contamination of drought-stressed peanut grown at Yuma, Arizona in 1992 with and without subsurface irrigation.

		Aflatoxin contamination			
Subsurface	No. of	Mean	C.V.	Escapes[1]	
irrigation	samples	(ppb)	(%)	Number	%
Yes	96	19,143	120	4	4
No	49	4,174	339	20	41

[1]Escape = sample with < 5 ppb.

subjected to a summer drought stress were escapes (Table 3).

A similar advantage from the use of subsurface irrigation was observed in 1992 (Table 6). The use of subsurface irrigation in 1992 increased the mean contamination by over 100%, reduced the C.V. by over 50%, and reduced the percentage of escapes by over 90% when compared to no subsurface irrigation.

Although the use of subsurface irrigation in this desert environment resulted in a substantial reduction in the C.V. associated with PAC, it is still large relative to variability associated with most other traits of interest. PAC is a highly variable trait in peanut. The development of new or refined screening techniques which can be used to further reduce this variability would help to maximize future breeding progress in developing resistance. However, current breeding progress should not be precluded by the level of variability associated with PAC using present field screening techniques.

Conclusion

In summary, this work demonstrated that preharvest aflatoxin contamination will occur in peanut subjected to a late summer drought stress at Yuma, Arizona. The use of subsurface irrigation to prolong plant viability during the drought stress resulted in higher and more consistent contamination. This system can be used to conduct large-scale field screening of peanut germplasm for resistance to preharvest aflatoxin contamination.

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