EXPERIMENT 1

Effects of root exudate, drought, and peanut genotype on *Aspergillus flavus* populations

INTRODUCTION

Throughout most of the world's peanut (*Arachis hypogaea* L.) production zone, *Aspergillus flavus* Link ex. Fires and related fungi are present in soils and may infect peanut pods. Hot and dry soil conditions favor high levels of *A. flavus* colonization of peanut fruits and aflatoxin production (Diener *et al*., 1987; Cotty *et al*., 1994). Colonization of peanut seeds is inversely proportional to maturity and is greatest with elevated geocarposphere temperature (Sanders *et al*., 1981). The daily mean temperature in the geocarposphere that is required for aflatoxin development during the latter part of the peanut growth cycle was found to be between 25.7 °C and 27 °C (Blankenship *et al*., 1984), whereas a daily mean geocarposphere temperature of 29°C led to the greatest levels of aflatoxin contamination in pods (Hill *et al*., 1983).

Initial infection by *A. flavus* may occur through the peg or during pod development (Haixin *et al*., 2000). Frank *et al*. (1994) found that soil-borne fungi in field were distributed within the horizontal fruiting zone, with a moderate to high degree of aggregation of fungi near pods. The mixing effects of plowing or disking a field may help break up fungal aggregates and distribute *A. flavus* propagules, possibly diluting these higher population densities (Griffin *et al*., 2000).

Under drought, the intensity of fungal invasion of pods and seeds differs among genotypes. It is possible that *A. flavus* uses exudates or sloughed cells that are near the surfaces of roots and pods as growth substrate. Peanut roots also exude soluble compounds that affect germination of fungal spores (Griffin, 1973). Sloughed root cells and soluble root exudates are the principal sources of organic carbon for microorganisms colonizing the rhizosphere of plant, especially supported *A. flavus* development on peanut roots and pods. Amounts of exudates are likely to differ

among genotypes and have been shown to increase in response to stress for barley (*Hordeum vulgare* L.) (Fan *et al*., 1997) and corn (*Zea mays* L.) (Young *et al*., 1998). Ricdel (2003) reported that drought increased root exudates, which then became growth substrates for soil-borne plant pathogens (fungi, bacteria, nematode). Yingthongchai (1994) reported that the flowering stage was the most critical growth stage for *A. flavus* infection of peanut but degree of infection varied with level of resistance among genotypes.

Because peanut grown under drought is often contaminated with aflatoxin, one possible avenue to avoid aflatoxin contamination would be to use drought-resistant variety as a proxy for screening peanut genotypes for aflatoxin resistance (Azaizeh *et al*., 1989). Drought-resistant genotypes are able to maintain internal plant water status for normal metabolic functioning with a limited water supply (Subbarao *et al*., 1995). Particularly important for drought resistance is a deep root system, which can absorb water from deeper soil layers.

Several laboratory methods have been tested to screen peanut genotypes which are resistant to *A. flavus* infection or aflatoxin production (Mehan and McDonald, 1980). Genotypes with aflatoxin resistance have either resistance to *A. flavus* infection, or prevention of aflatoxin production, or both. Efforts to develop aflatoxin-resistant peanut germplasm, however, have made only modest progress (Holbrook *et al*., 1994). Peanut pods that are damaged or seeds that are discolored are generally infected with aflatoxin and may be easily removed, so by using the term of aflatoxin resistance, it refers only to the peanut pods and seeds (Wilson *et al*., 1977).

Mixon and Roger (1973) and Mehan and McDonald (1980) developed inoculation methods for screening peanut genotypes using rehydrated, mature, sound

seed with assay of aflatoxin contamination by thin layer chromatography. After harvest, seed testa were characterized for their cell structure, cell arrangement, permeability, waxy surface and tannin content, all factors were found to be associated with resistance to fungal colonization (Mixon, 1980). Zambettakis *et al*. (1977) reported that, in general, peanut genotypes were infrequently infected in both laboratory and field samples. Progress in developing cultivars with resistance to aflatoxin contamination has been slow, both because infection and contamination levels show great variability and because there is little correlation between results of laboratory screening techniques and aflatoxin contamination under field conditions (Blankenship *et al*., 1985) or under storage conditions (Wilson *et al*., 1977).

Ingram *et al*. (1999) showed that a minirhizotron can be used both to observe growth and development of peanut pod and infection of pods and roots by *A. flavus* that produces a green fluorescing protein (GFP) that can be seen on peanut roots and pods *in situ*. This technology is a non-destructive method of observing pod growth and *A. flavus* infection *in situ*. Thus, this experiment combines these technological advances and applies them to observe the distribution of *A. flavus* population in the soil and relationship with drought stress.

The objectives of this study were: 1) to observe *A. flavus* growth on root and pod surface under soil surface in response to water deficit; 2) to examine genotypic differences in *A. flavus* colonization; and 3) to evaluate the effect of exudates or leachates on *A. flavus* population in soil.

MATERIALS AND METHODS

Greenhouse research was conducted at the Georgia Envirotron, at the Griffin Campus of the University of Georgia, USA during 2001, and during 2003 at the Lampang Agricultural Research and Training Centre, Lampang, and at the Faculty of Agriculture, Chiang Mai University, Thailand.

Sub-experiment 1. Observation of *Aspergillus flavus* population on the root and pod zone

Four peanut genotypes (329CC: aflatoxin resistant; 419CC: drought and aflatoxin susceptible; 511CC: drought and aflatoxin resistant (Holbrook *et al*., 1993); and Georgia Green: commercial variety from the Southeastern USA) were grown in 214-liter containers with sandy soil (Tifton loamy sand) collected from the Blackshank Farm, Tifton, Georgia (Appendix B). Soil composition was 86% sand, 8% clay and 6% silt. Each container consisted of four plants with one container taken as one replication. Containers were fitted with minirhizotron observation tubes, installed horizontally at 5, 25, 50 and 75 cm below the soil surface. Half of the containers had moisture blocks and thermocouples installed at 5, 25 and 75 cm depth. Each of these environmental parameters was recorded with a CR10X data logger and stored as hourly average through the experiment. All containers were irrigated lightly at 1- to 2-day intervals until seedlings established. At 14 days after planting (DAP), all containers were irrigated with modified half-strength Hoagland's solution, whose composition is shown Table 1.1.

At 25 DAP, two water treatments were imposed: 1) well watered though irrigation to field capacity twice weekly; and 2) multiple water-deficits imposed in

three-week cycles by withholding irrigation for two weeks followed by irrigating twice during the third week, for a total of four treatment cycles.

Table 1.1 Nutrient components of half strength Hoagland's solution for peanut culture.

Stock A (Liter)		Stock B (Liter)		A5 stock (Liter)		$FeEDTNa_2$ (Liter)	
Compound	Amount	Compound	Amount	Compound	Amount	Compound	Amount
KH_2PO_4	13.6 g	$FeEDTANa_2$	100 ml	$CuSO_4.5H_2O$	0.0786 g	$EDTANa_2.2H_2O$	33.4 g
KNO_3	50.6 g	$Ca(NO_3)_2.H_2O$	118 g	$MnSO_4.H2O$	1.54 g	$FeSO_4.7H_2O$	24.9 g
$MgSO_4,7H_2O$	49.3 g			$ZnSO_4.7H_2O$	0.22 g		
NaCl	5.8 g			H_3BO_3	2.86 g		
A5 stock	100 ml			H_2MoO_4	0.0176 g		

Half strength Hoagland's solution was made from 5 ml of stock A and 5 ml of stock B in 1 liter water.

Inoculum preparation and application

At 30 and 40 DAP, the soil surface of all containers was inoculated with cracked corn inoculum which had been cultured with a strain of GFP *A. flavus* obtained from Jeffery Carey (USDA-ARS, New Orleans, LA). The GFP *A. flavus* was cultured on Petri dishes containing M3S1B medium, an *A. flavus-A. niger* group selective medium. The medium was a 2,6-dichloro-4-nitroaniline–amended medium (10 mg/liter) originally developed by Bell and Crawford (1967) and modified by Griffin and Garren (1974). M3S1B medium had following composition: 5.0 g peptone, 10.0 g glucose, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 30.0 g NaCl, 20.0 g agar, 50.0 mg streptomycin sulfate, 50.0 g chlorotetracycline, 1.0 mg 2,6-dichloro-4-nitroaniline (added in 3 ml acetone) and 1 liter distilled water. When spores had been formed, they were washed from the mycelia with 50 ml sterile deionized water and stored in a refrigerator at 3 to 5 °C.

Corn (*Zea mays* L.) seeds were coarsely ground in a blender to make cracked corn. Then 200 g of cracked corn was placed in each of four stoppered 250 ml flasks and autoclaved twice. After the cracked corn had cooled, a 50 ml aliquot from spore strain was added to each flask and incubated at 30 °C for 5 days. After 5 days, this cracked corn inoculum was applied immediately on the topsoil surface by spreading and mixing with soil as on the line of minirhizotron tubes.

At 7 weeks after inoculation (74 DAP), a minirhizotron camera (Appendix A) was used to observe GFP *A. flavus* population on surface of root, pegs and pods at the 5 cm depth by intensity of fluorescence when illuminated with UV light. Root and pod images were recorded with a minirhizotron camera directly to a laptop computer hard drive (Patena and Ingram, 2000).

All images were analyzed for green fluorescence color of *A. flavus* fungus by Quantitative Analysis of Color System (QuaCos) program. QuaCos is a program written in visual basic to analyze red, green and blue values of a pixel or group of pixels in a digital image and produces a spreadsheet of values for color intensities. Because GFP *A. flavus* fluoresces green, I analyzed only green color values. Green color intensity was scored on a 0 to 255 scale. Full minirhizotron images were analyzed in 20×20 pixel groups. The total number of group was analyzed 32×24 groups for each image that would be the total relative intensity of GFP *A. flavus*. The program stored color values in a text file. Data were graphed using Microsoft Excel™ Surface Chart type with the contour-color option. Data were also analyzed numerically, based on the total color intensity for each image (Appendix C).

Sub-experiment 2. Peanut root exudate effect on *Aspergillus flavus* population

Seeds of four peanut genotypes (419CC: drought and aflatoxin susceptible; 511CC: drought and aflatoxin resistant; Tainan 9: commercial variety in Thailand; and Luhua 11: aflatoxin resistant from China) were surface sterilized with 10% Clorox for 60 s, then rinsed twice with sterile water. Seeds were pre-germinated on moist paper for 3 to 5 days. Uniform seedlings were transplanted into a hydroponic system with half-strength Hoagland's solution. Each container consisted of 4 plants (one container as one replicate).

The experimental design was a 4 × 2 factorial in a randomized complete block with three replications. Treatments were: four peanut genotypes and two levels of water stress (control and impose polyethyleneglycol (PEG)). Containers were fitted with an automatic pump system for aeriation (Figure 1.1). All hydroponic containers

were placed in a green house during 14 May to 15 June 2003. Average green house conditions were: 37.15°C maximum temperature, 27.45°C minimum temperature; 76.94% maximum RH, 55.64% minimum RH; light intensity 39.5 mol/m^2/day (Appendix D).

Every 7 days, nutrient solution was replaced with fresh solution. At 32 DAP (flowering stage), water stress was imposed in half of the hydroponic containers by adding PEG 4000 to the nutrient solution (50 mg PEG/liter nutrient solution) for 24 hrs.

Four whole plants were removed from each container and their roots gently rinsed with tap water. Root systems were transferred to a beaker, containing 500 ml deionized water for 24 hours. After 24 hr, 200 ml root zone solution was collected from each beaker and stored in 200-ml plastic bottles at -20 °C. Peanut plants were then separated into shoot and root portions, shoots were dried at 80 °C for 48 hours and roots were stained with methyl violet (2.5 g methyl violet/250 ml 95% ethanol then diluted to 1 ml methyl violet solution/1 ml H_2O).

Two *A. flavus* strains were separately cultured on M3S1B medium until they produced spores. Spores were washed from the cultures with distilled water producing a spore suspension with 6.05×10^6 spore/ml as estimated by counting the spores under a microscope with a hemacytometer. The two *A. flavus* strains were modified to produce a green fluorescence protein, one strain was produced by Gary Payne (NCSU, Raleigh, North Carolina), the other by Jeffrey Carey (USDA-ARS, New Orleans, Louisiana).

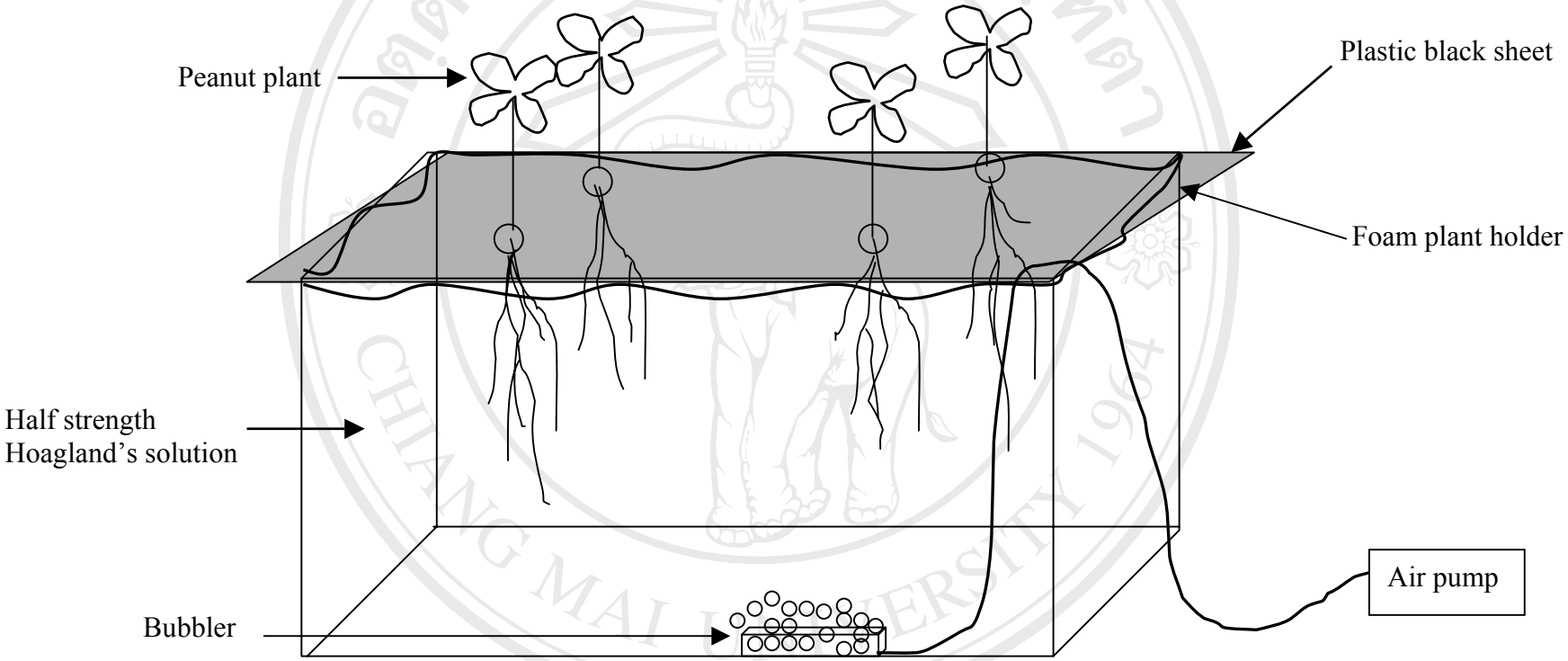

Figure 1.1 Diagram of hydroponic system for peanut cultivation in green house.

River sand from Chiang Mai, Thailand was steam sterilized at 110-130 °C for 4 hrs. After cooling 20 g of the sterile sand was placed in a Petri dish into which 1 ml of *A. flavus* spore suspension was mixed, then 5 ml of root zone solution from peanut plants growing in hydroponic culture was also added. Petri dishes were incubated in the dark on a bench top at about 30 °C for 5 days. After incubation, GFP *A. flavus* population in the sand culture was observed as follow:

All soil from a Petri dish was placed in a Waring blender and mixed with 200 ml sterile water at low speed for 60 s. The mixed solution was diluted to 10^{-4} and 10^{-5} concentration. After dilution, 0.5 ml of soil solution was spread on three Petri dishes with M3S1B medium. Plates were incubated for 5 days at 30 °C before counts of *A. flavus* group colonies. Results were recorded as colony-forming units of *A. flavus* group/gram soil.

Stained root systems were analyzed for the total root length, root surface area, root volume and average root diameter, using a flatbed scanner and WinRhizo software (V. 4.0 B, Regent Instrument Inc., Quebec, Canada).

Sub-experiment 3. Exudates and sloughed cells from peanut roots in soil culture

The same four genotypes as in the sub-experiment 2 were grown in plastic containers (diameter 15 cm) with river sand soil that had been steam sterilized at 110-130 °C for 4 hrs. This experiment was conducted at Faculty of Agriculture, Chiang Mai University. All containers were placed outside under ambient conditions. The experiment was comprised of 4 replications (3 plants per container as one replication) of four genotypes. A control treatment with containers of sterilized sand without peanut plants was also maintained. All containers were irrigated at 1- to 2-day intervals with half-strength Hoagland's solution.

At 30 DAP (flowering stage), 20 g of sand was sampled from the upper 5-cm layer of all containers, mixed with 1 ml of spore suspension of GFP *A. flavus* (5.89×10^5 spore/ml), placed in a Petri dish, and incubated at 30 °C for 5 days. After incubation, all soil from a Petri dish was placed in a Waring blender and mixed with 200 ml sterile water at low speed for 60 s. The mixed solution was diluted to 10^{-4} and 10^{-5} concentration. After dilution, 0.5 ml of soil dilution was spread on each of two Petri dishes with M3S1B medium. Petri dishes were incubated for 5 days at about 30 °C. Populations of *A. flavus* in sand were recorded as colony-forming unit/gram sand.

Statistical analysis

Data were analyzed by the general linear model procedure of SXW (Statistix For Windows; Analytical Software, Tallahassee, FL), and SAS statistical package, Version 7 (SAS Institute, Cary, North Carolina, USA). Means were compared by least significant difference (LSD). Unless otherwise stated, all significant differences were tested at $P \leq 0.05$.

RESULTS

Aspergillus flavus population on the root and pod zone under water deficit.

Soil moisture potential in the multiple water deficit treatments are shown in Figure 1.2. Water potential measurements at 5 cm depth showed that this level was drier than 25 and 75 cm depths, with drought condition suitable to favor *A. flavus* colonization.

Minirhizotron observations at 5 cm soil depth showed that GFP *A. flavus* colonized roots and pods (Figure 1.3). By QuaCos analysis, *A. flavus* populations were relatively constant in the root surface as 20-50 and pod surface as 10-25 scale of green color values (Figure 1.4). Data for colonization of roots by *A. flavus* are shown in Table 1.2. Genotypes 419CC and 511CC had greater GFP *A. flavus* populations on roots than genotypes 329CC and Georgia Green. Most genotypes had larger *A. flavus* populations on roots under water deficit than under well-watered conditions. The exception was 329CC which had a greater density of fungi on roots under well-watered conditions. At 5 cm soil level, the root of 329CC and 419CC genotypes were able to extract high soil moisture. Moreover, the root of 329CC could also extract more soil moisture at 25 and 75 cm depth of soil than 419CC.

Among the four cultivars, Georgia Green had the highest soil water potential at 5 cm depth, that is, it extracted the least amount of moisture at this depth. On the other hand, Georgia Green extracted the moisture to the lowest soil water potential at 25 and 75 cm depths. Moreover, roots at 5 cm soil layer of Georgia Green highly increased the colonization of *A. flavus* by 55% under water deficit condition. Even with soil water potential of -1.457 MPa at 5 cm depth for Georgia Green, water deficit was sufficient to increase *A. flavus* population.

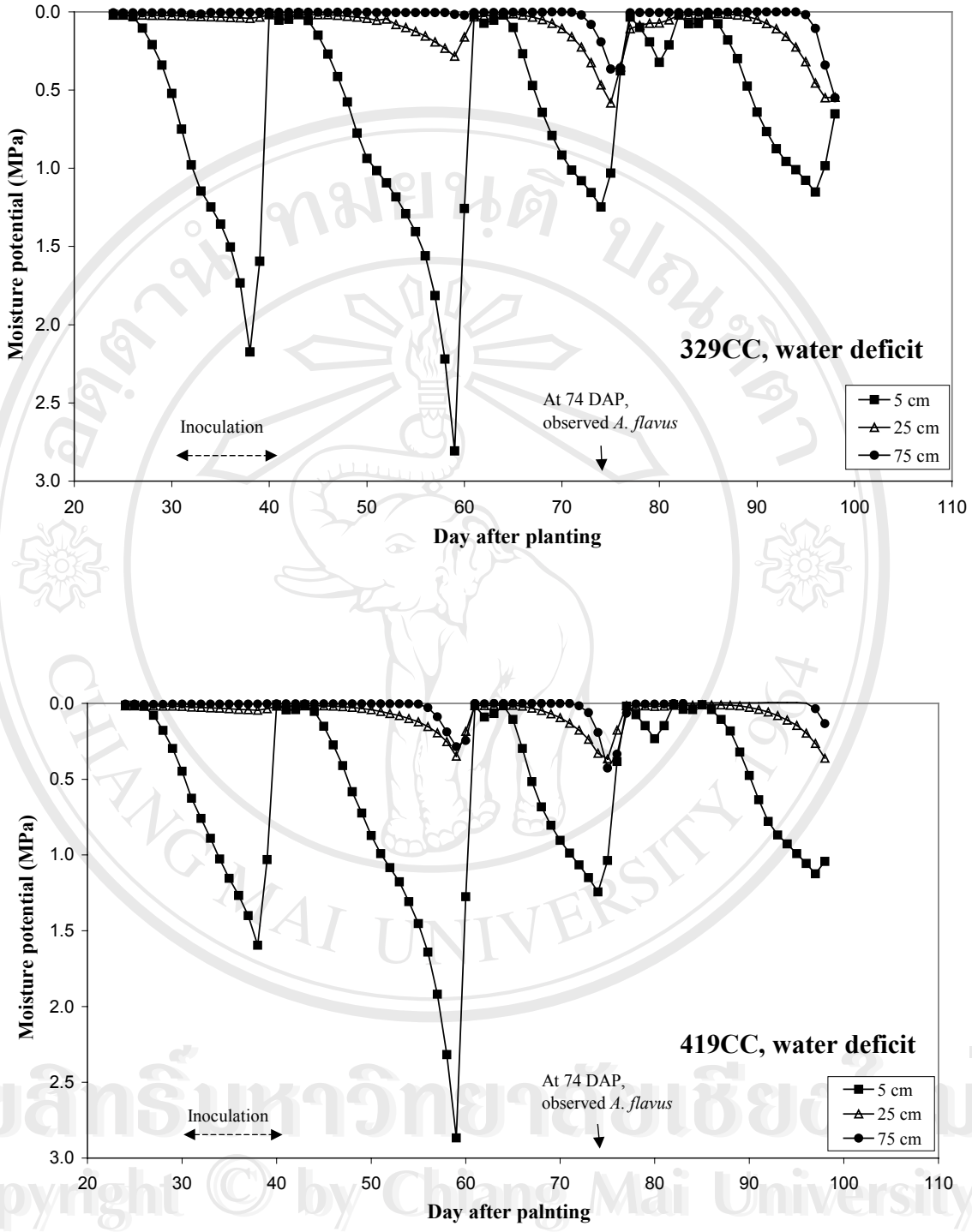

Figure 1.2 Soil moisture potential at three depths through four water deficit cycles for four peanut genotypes.

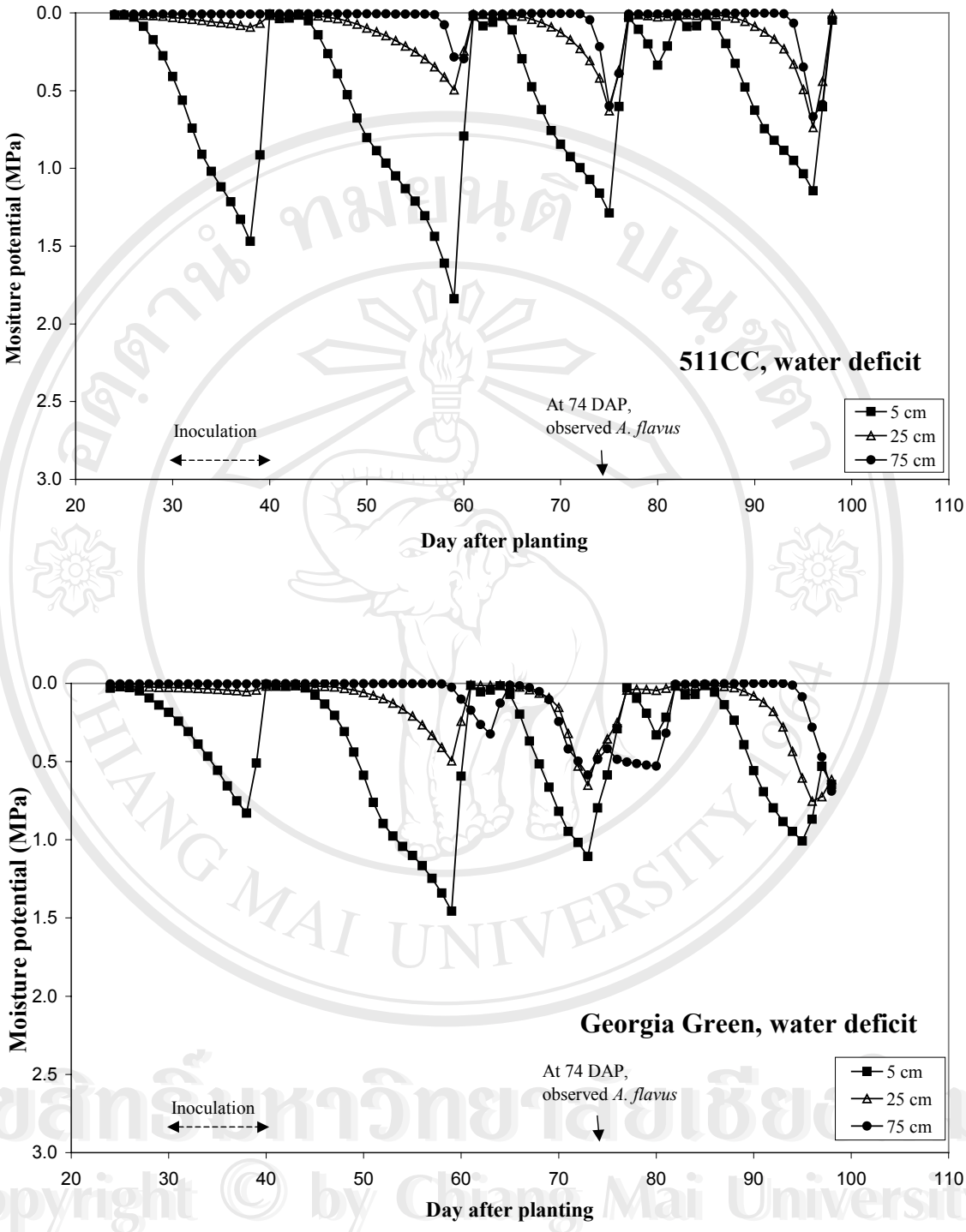

Figure 1.2 continued

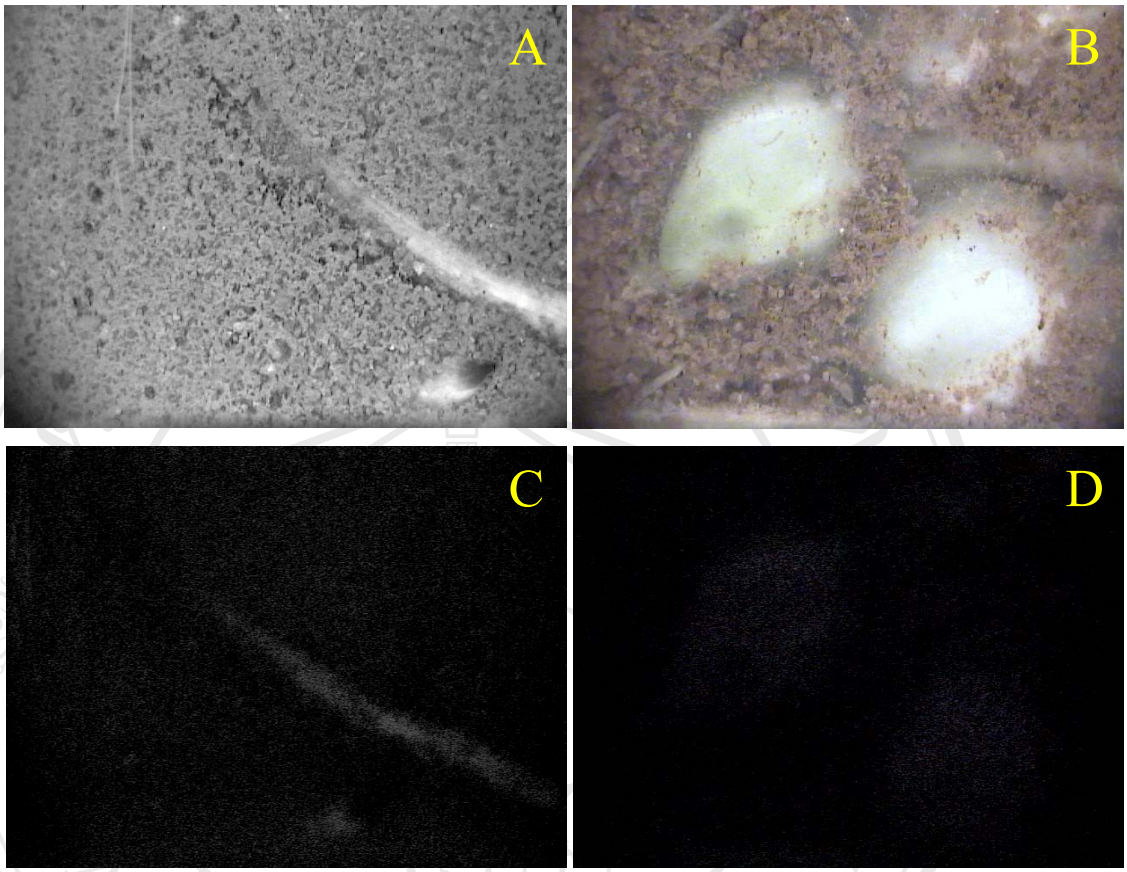

Figure 1.3 Root (A, C) and pod (B, D) images as observed by a minirhizotron camera: (A-B) observed with a white light; (C-D) observed with UV light. Fluorescence in C follows the large root, with additional patches of fluorescence near to the right of center near the bottom of the image and scattered fluorescence near the center above the root. Diffused fluorescence in D is barely visible to the naked eye just above and to the left of the center of the image and in the lower right quadrant.

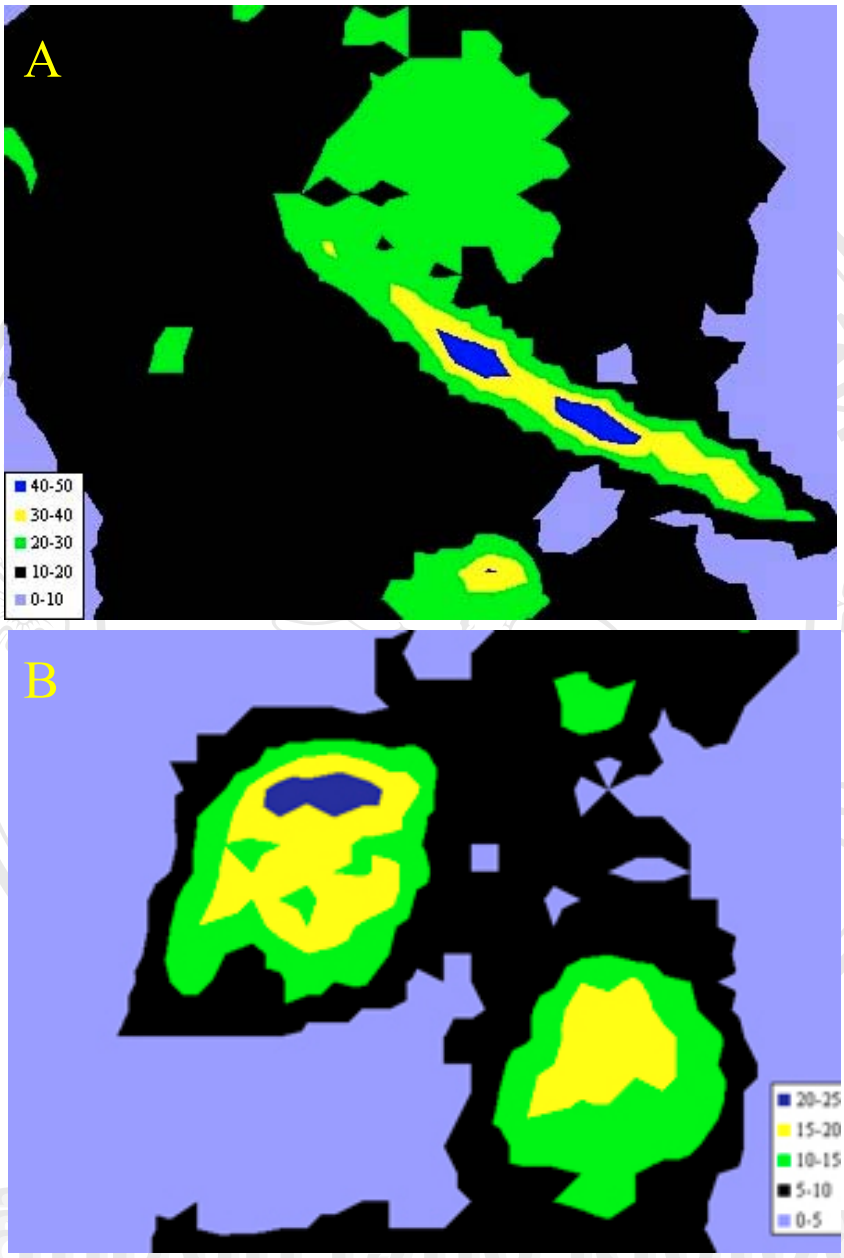

Figure 1.4 *Aspergillus flavus* population density on roots and pods as estimated by QuaCos program. (A) *A. flavus* colonies on roots; (B) *A. flavus* colonies on pods.

Table 1.2 Relative *Asperillus flavus* population density (32×24 groups of each image) on the root zone at soil 5 cm depth layer of four peanut genotypes grown under water deficit and well-watered condition at the Georgia Envirotron.

Genotype	Water treatment		Mean
	Water deficit	Well- watered	
	Relative green fluorescence color value, image total		
329CC	9,284	10,078	9,681 bc*
419CC	17,420	9,914	13,667 a
511CC	11,799	10,436	11,118 ab
Georgia Green	10,624	4,769	7,696 c
Mean	12,392 a	9,068 b	10,730

* Means followed by the same letter within a column or row are not significantly different at P = 0.05 by LSD

A. flavus population densities in the pod zone are shown in Table 1.3. The GFP *A. flavus* colonized in the pod zone (Figure 1.3B-D), especially on pod surface (Figure 1.4B). Genotype 419CC had the greatest population of *A. flavus* in the pod zone, moreover the *A. flavus* densities of this genotype were not significant differences between water deficit and well-watered. Most genotypes had greater densities of fungal population in the pod zone under water deficit than under well-watered conditions. Water deficit had increased *A. flavus* population in pod zone of genotype 511CC by 65%.

Though data cannot statistically compare *A. flavus* population densities in root (Table 1.2) and pod zones (Table 1.3), *A. flavus* populations were all at least three times higher in the root zone than in the pod zone, with some treatments having more than 6 times as much *A. flavus* in the root zone than in the pod zone.

Table 1.3 Relative *Aspergillus flavus* population density (32×24 groups of each image) on the pod zone at soil 5 cm depth layer of four peanut genotypes grown under water deficit and well-watered condition at the Georgia Envirotron.

Genotype	Water treatment		Mean
	Water deficit	Well- watered	
	Relative green fluorescence color value, image total		
329CC	1,936	1,538	1,731 c
419CC	2,954	3,065	3,009 a*
511CC	2,928	1,776	2,352 b
Georgia Green	2,667	2,212	2,437 ab
Mean	2,617 a	2,143 b	2,380

* Means followed by the same letter within a column or row are not significantly different at P = 0.05 by LSD

Effect of root exudate on *Aspergillus flavus* populations

Stored root exudate solution from peanut root of each genotype under hydroponic system cultured with GFP *A. flavus* fungi indicated that *A. flavus* fungi had high level of colonization but it occurred less than the stock of spore suspension (stock: 6.05 x 10^6 spores/ml). The *A. flavus* populations supported by root exudates of each peanut genotype are shown in Table 1.4. Genotype 419CC and Luhua 11 had highest level of *A. flavus* colonization by root exudate supporting. Root exudates from peanut plants in the water deficit treatment had increased *A. flavus* populations, except Tainan 9.

Total root length showed significant differences between genotype, and Tainan 9 genotype was higher in root length than 511CC and Luhua 11 genotypes (Table 1.5). Genotype 419CC had the large total root length of 6.54 m/plant, contrary, 511CC and Luhua 11 had small root length of 5.28 and 4.82 m/plant, respectively. However, root exudates of Luhua 11 had highly increased *A. flavus* growth. On the other hand, root exudate of 511CC had small effect to increase *A. flavus* population. Under hydroponic system, the small root length of peanut genotypes was higher to support the substrate of exudate from the root cell to *A. flavus* fungi than the large root length genotype, except 419CC genotype.

Table 1.4 Colonization of *Aspergillus flavus* cultured with the root exudate solution of four peanut genotypes grown under hydroponic system, which induced water deficit condition by imposing polyethylene glycol for 24 hrs and no polyethylene glycol.

Peanut genotype	Treatment		Mean	
	Control	PEG		
	A. flavus colonies x 10^4 /gram sand †			
419CC	11.7±0.1	13.7±2.3	12.7±1.3	ab*
511CC	9.33±2.6	13.0±1.2	11.2±1.5	bc
Luhua 11	13.0±1.2	16.7±2.0	14.8±1.3	a
Tainan 9	9.00±1.2	9.00±1.5	9.00±0.9	c
Mean	10.8±0.9	13.1±1.1	11.9±0.7	

† Mean ± standard error of mean

* Means followed by the same letter are not significantly different at P = 0.05 by LSD

Table 1.5 Total root length of four peanut genotypes grown under hydroponic system, which induced water deficit condition by imposing polyethylene glycol for 24 hrs and no polyethylene glycol. Root length measured by a flatbed scanner and WinRhizo.

Peanut genotype	Treatment		Mean	
	Control	PEG		
	Total root length (m/plant)			
419CC	6.51	6.57	6.54	ab*
511CC	5.27	5.29	5.28	bc
Luhua 11	5.53	4.10	4.82	c
Tainan 9	7.31	7.61	7.46	a
Mean	6.15	5.89	6.02	

* Means followed by the same letter are not significantly different at P = 0.05 by LSD

***Aspergillus flavus* growth in soil culture with peanut plants.**

For *A. flavus* cultured on sand in which peanut plants had previously grown, soil for genotype 419CC had 12.3 x 10^4, Luhau 11 had 10.0 x 10^4, Tainan 9 had 9.42 x 10^4 and 511CC had 9.33 x 10^4 *A. flavus* colonies/gram sand. The control treatment had 5.75 x 10^4 *A. flavus* colonies/gram sand which was significantly less than sand on which peanut plants had grown, but there were no significant effects of peanut genotypes on *A. flavus* populations in the sand media.

DISCUSSION

Aspergillus flavus colonization on roots and pods at 5 cm soil depth layer

By minirhizotron and subsequent QuaCos analysis, I was able to observe colonization of *A. flavus* on peanut roots and pods *in situ*. Water deficit increased GFP *A. flavus* population density on roots and pods. The fluorescence of GFP *A. flavus* as directly observed with UV light is not visible to naked eye, but QuaCos allows me to detect this fluorescence.

At 5 cm depth soil, 329CC used the most soil moisture (Figure 1.2), but 329CC had less *A. flavus* colonization in the pod zone. Drought-susceptible 419CC had relatively high *A. flavus* population on pods. Sanders *et al*. (1993) reported that drought in the pod zone is necessary and adequate for aflatoxin contamination. More aflatoxin contamination was found in peanut that grew under drought than irrigated peanut (Pettit *et al*., 1971) and peanut seed was more susceptible to *A. flavus* infection when soil moisture levels in the pod zone were low enough to reduce seed moisture below 31%. On the other hand, 511CC used less soil moisture at 5 cm than did 329CC and 419CC. Genotype 511CC appears to be drought resistant, but has relatively high *A. flavus* population in the pod zone under water deficit conditions. It is possible that 511CC and 419CC produce more root exudates which are substrates for *A. flavus* (Griffin *et al*., 1975).

A. flavus population densities were greater on roots (Table 1.2) than on pods (Table 1.3). Roots may slough more cells and exude more soluble substrates than pods, especially glucose (Pass and Griffin, 1972), sucrose and fructose (Hale and Griffin, 1976), thereby allowing greater *A. flavus* development on peanut roots than on pods (Table 1.4).

Preharvest aflatoxin contamination of peanut occurs under heat and drought stress (Figure 1.2). Under water deficit, all peanut genotypes had greater densities of fungal populations on pods than under well-watered conditions, especially the drought-susceptible genotype 419CC. Dorner *et al*. (1989) reported that root of stressed plants would not be able to supply sufficient moisture to pods, thus pods would be susceptible to infection. *A. flavus* populations appeared to be greater on roots and pods of drought-susceptible peanut genotypes than on roots and pods of drought-resistant genotypes (Ingram *et al*., 1999). Peanut genotypes, low in both spore germination and colonization density of *A. flavus*, should be preferred as more promising potential of aflatoxin resistance (Frank *et al*., 1994).

The green house experiment confirmed that water deficit increased *A. flavus* populations, and that *A. flavus* populations differed among genotypes. This research suggests that the drought susceptibility of a peanut genotype is related to colonization of *A. flavus,* but shows clearly that *A. flavus* populations are not related to the apparent drought or aflatoxin resistance of genotypes. Even cultivars with relative drought and aflatoxin resistance supported large *A. flavus* colonies in the pod zone.

Root solution effect on *A. flavus* spore germination

Under water deficit, root cell membranes lose their integrity and roots leak more soluble substrates (Premachandra and Shimada, 1987). *A. flavus* populations were greatest when cultured with root exudate solutions of Luhua 11 (Table 1.4) which suggested that this genotypes released more growth substrates for *A. flavus* than did the other genotypes. The mean *A. flavus* population in sand treated with root solution exudates was 1.19×10^5 colonies/gram sand which was less than numbers of

spores added in the suspension stock, 3.02×10^5 spores/gram sand. The suspension stock probably had both viable and non-viable spores, so only a portion of the spores could germinate. Substrates in root growth solutions might also have been so small as to support only minimal *A. flavus* growth. Additional research is needed to analyze the substrates and their concentrations in the root growth solutions. The fact that *A. flavus* populations cultured with root exudate solutions did not absolutely reflect differences in drought or aflatoxin resistance suggests that root exudates probably is not a trait suited for aflatoxin resistance screening.

Genotypes differ in root size, morphology and physiology (Marschener, 1998). In particular, a large root surface area might produce more root exudate or sloughed cells that enhanced rhizosphere microorganism growth. In turn, rhizosphere microorganism can either enhance or depress root growth in general or the formation of root hairs (Marschener, 1995). In hydroponic culture, 419CC had the greatest root length, which, if exudation is related to root area, may explain the high rate of colonization by *A. flavus*. In contrast, Luhua 11 had relatively high colonization by *A. flavus* but small root length.

Certainly, the amount of root exudate is not always related to total root length. Both Tainan 9 and 419CC had large root length but the root exudate solution of Tainan 9 had no effect on *A. flavus* population. This result suggests that the amount and composition of root exudates differ among genotypes. Whipper (1990) reported that the amount and composition of root exudate is variable, depending on plant genotype and environmental conditions such as CO_2, light, temperature, moisture and nutrients.

Under water deficit, peanut roots slough root cap cells, cortical tissue sheet, and tissue fragments. Some roots also die. All of these dead cells can affect soil microorganism activity (Bolton *et al*., 1992). Dead root cells and leachates are substrate for *A. flavus* that is capable of growing and reproducing on media containing carbon source. Soil in which peanut plant had grown certainly contained more dead root cells or leachates than soil without peanut plants, as shown by *A. flavus* colonization in sub-experiment 3.

A minirhizotron system allows *in situ* observations of GFP *A. flavus* colonization of peanut roots and pods. Fluorescence was often adjacent to root or pod surfaces which suggests that *A. flavus* was not infecting the living tissue, but rather grew on exudates or sloughed cells adjacent to the roots and pods. Most important, the combination of minirhizotron, GFP *A. flavus,* and Quacos was relatively easy to use to estimate *A. flavus* population densities under natural soil condition, and less time-consuming than laboratory screening. Though neither *in situ A. flavus* populations nor *A. flavus* populations cultured from root exudates were related to apparent drought or aflatoxin resistance, it suggests that these are not observations suited to aflatoxin screening, but they remain valuable methods to enhance understanding of plant-fungal interactions.