SUMMARY AND CONCLUSIONS

By using minirhizotron observations at 5 cm soil depth and subsequent QuaCos analysis, I was able to observe colonization of *Aspergillus flavus* on peanut roots and pods *in situ*. Data for colonization of roots by *A. flavus* are shown in Table 1.2. *A. flavus* population densities in the pod zone are shown in Table 1.3. Minirhizotron observations and QuaCos analysis showed that GFP *A. flavus* colonized roots and pods (Figure 1.3) and *A. flavus* populations were relatively constant in the root and pod surface (Figure 1.4). The fluorescence of GFP *A. flavus* as directly observed with UV light is not visible to naked eye, but QuaCos made the detection of this fluorescence possible.

At 5 cm soil level, water deficit increased GFP *A. flavus* population density on roots and pods. Genotypes 419CC and 511CC had greater GFP *A. flavus* populations on roots than genotypes 329CC and Georgia Green. The root of 329CC and 419CC genotypes were able to extract high soil moisture. On the other hand, 511CC used less soil moisture at 5 cm than 329CC and 419CC genotypes. Genotype 511CC appears to be drought resistant, but has relatively high *A. flavus* population in the pod zone under water deficit conditions. Water deficit had increased *A. flavus* population in pod zone of genotype 511CC by 65%.

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.

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 resistant genotypes. Even cultivars with relative drought and aflatoxin resistance supported large *A. flavus* colonies in the pod zone.

In hydroponic culture, *A. flavus* populations were greatest when cultured with root exudate solutions of Luhua 11 (Table 1.4). Genotype 419CC and Luhua 11 had highest level of *A. flavus* germination by root exudate supporting. Root exudates from peanut plants in the water deficit treatment had increased *A. flavus* populations, except Tainan 9. Genotype 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.

A. flavus cultured on the sand without peanut plants have less population than on which peanut plants was grown. Soil in which peanut plant was grown certainly contained more dead root cells and leachates than in the soil without peanut plants as shown by *A. flavus* colonization.

This experiment conclude that 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 was related to apparent drought or aflatoxin resistance, it suggests that these are not observations suited for aflatoxin screening, but they remain valuable methods to enhance the understanding of plant-fungal interactions.

In experiment 2, the spraying of spore suspension of GFP *A. flavus* to the plant shoot, especially on flowers led to high levels of *A. flavus* infection at the flowering and pegging stages of the peanut plant. Inoculum of *A. flavus* applied to the soil surface also led to high levels of peg infection (Table 2.1). These inoculating methods were artificial treatments that had certainly infected on the peanut by *A. flavus* fungi. By observation with a UV microscope at 5 days after inoculation, GFP *A. flavus* was found on the surface of peanut flowers (Figure 2.2A-B) and the hyphae of GFP *A. flavus* penetrated into the flower tissues. Embryos in some dissected pegs was fluoresced (Figure 2.3A), indicating internal colonization by GFP *A. flavus*.

By spraying plants with a spore suspension, the percentage of infection was higher than without spraying. Fungal spores attached to the tips of stigma with pollen grain (Figure 2.5A-B), follow the path of pollen. Fungal spores lodge on the stigma and germinate, following the pollen tube as it enters the style, then infects the ovary. As gynophore elongates, the fungus remains with the ovary, becoming established in developing seeds. In this study, *A. flavus* infected peanut flowers and pegs, which explains how *A. flavus* can infect seeds without damaging the pods.

A high soil-surface population of applied toxigenic strains had affected the flower infection with subsequent aflatoxin contamination. Under open-field conditions, the highest infection of floral tissues was found on the treatment with

cracked corn inoculum applied to the soil surface (Table 2.3). The soil was likely already colonized by *A. flavus*, which could produce spores capable of infecting peanut flowers. Therefore, the combined applications of a spore suspension over shoots with flowers and a cracked corn inoculum applied to the soil should result in the greatest *A. flavus* infection levels.

In experiment 3, water deficit did not decrease main stem elongation or individual leaf area of 329CC until harvest, indicating that 329CC was able to maintain shoot and leaf growth during water deficit and recovered quickly when water was available. Under water deficit, 329CC extracted the most soil moisture at 5 cm depth, and was highly capable of using the moisture for pod yield production (Table 3.3). The root system of 511CC extracted the most moisture throughout the soil profile, suggesting that under water deficit it was able to maintain sufficient internal water status to continue its growth processes.

The root system of 419CC generally extracted the least soil moisture from 25 and 75 cm depths, indicating a relatively shallow root system. Genotype 419CC had a large main stem and large individual leaf area but the leaf moisture content was low.

The root system of Georgia Green extracted more soil moisture at 25 and 75 cm depth than 329CC and 419CC, which explains the greater pod yield of Georgia Green under water deficit.

The root system of 511CC allowed moisture uptake from deeper soil layers that improve drought avoidance by utilizing deeper soil water. However the root system of 329CC also maintained water balance under water deficit condition by extracting more soil water from the upper soil horizons but not able to reduce the relative yield loss. On the other hand, the root system of Georgia Green was low

distributed at shallow soil, and produced large root at deeper soil layer that may be able to absorb more moisture which can reduce the relative yield loss.

Although the drought-resistant genotype (511CC) was susceptible to *A. flavus* infection on the pod, seed infection was not enhanced by water deficit. Root systems of drought-resistant genotypes can extract soil moisture from both shallow and deeper soil layers, which can maintain moisture in the plant, thereby having less relative yield loss.

In conclusion, it was clear that under drought stress condition, the amounts of root and pod exudates are likely to differ among peanut genotypes. The genotypes that produce less root and pod exudates under water deficit condition provide less substrate for *A. flavus* growth so that smaller *A. flavus* populations are less likely to infect the low exuding genotypes. Neither in situ *A. flavus* population assessment nor *A. flavus* culture on root exudates appeared to be suitable traits for aflatoxin-resistant screening, however, they remain valuable method to enhance our understanding of plant-fungal interactions.

Peanut infection by *A. flavus* can occur during flowering stage and it appears that *A. flavus* hyphae follow the germinating pollen tube through the style to infect embryos. High infection of peanut pod (exterior) and shell (interior) by *A. flavus* were not related to seed infection. Genetic difference in susceptibility to drought stress in peanut was related to difference in the intensity of *A. flavus* infection.