# SUMMARY AND CONCLUSIONS

This study provided information on: i) combined effects of elevated atmospheric $CO_2$ and temperature on peanut growth and *A. flavus* infection, ii) differential plants response to water deficit and *A. flavu*s infestation by drought-tolerant and drought-susceptible peanut genotypes, and iii) pathway of *A. flavus* infection into peanut flowers related to preharvest infection.

In experiment 1, time from planting to flowering of Tainan 9 was influenced by temperature but not by $CO_2$. At 35/25 ºC, first flower occurred within 22 days, while at 35/25 ºC, it took 34 days. Significant effects of temperature and $CO_2$ were found in main stem length, individual leaf dry weight, total leaf area, SLA, and shoot and pod dry weight. Leaflet size was significantly affected by temperature but not by $CO_2$. Interactions between temperature and $CO_2$ were found in stem length, individual leaflet weight, and shoot dry weight. Neither temperature nor $CO_2$ significantly influenced branch number. Under two different air temperatures, peanut grown under increasing $CO_2$ from 400 to 800 µmol $mol^{-1}$ had positive effects on main stem length, above ground biomass by enhancing photosynthetic rate, whereas numbers of branches were similar among plants growing under those treatment combinations. At low temperature (25/15ºC), leaf area increased as $CO_2$ increased whereas specific leaf area declined. Minirhizotron observations showed that total root length, number of roots, and RLD were significantly greater for plants grown under 35/25ºC than 25/15ºC for all $CO_2$ levels Fibrous root dry weight was greater in plants exposed to

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35/25ºC than at 25/15ºC. Root dry weight increased with increasing $CO_2$ when plants grew at 25/15ºC but decreased as $CO_2$ increased at 35/25ºC.

In the rhizotron experiment, primary roots elongated to the bottom of rhizotrons by 13 to 14 days for plants grown at 35/25ºC and by 16 to 17 days for those at 25/15ºC. In general, a large proportion of root length and numbers were observed in the three upper soil layers (i.e. 0-100 mm, 100-200 mm, 200-300 mm) at low temperature, but only two upper soil layers at high temperature. Root length and root number at low temperature were greater than those at high temperature. Total root length significantly increased with increasing $CO_2$. There are conflicting results between short- and long-term responses of roots to $CO_2$ and temperature. The greater total root length at low temperature in the short-term experiment in rhizotrons may not necessarily indicate long-term responses. However, the thin-layer soil rhizotron system allows researchers to observe and quantify simultaneously the time courses of seedling root development without disturbing the soil or roots.

In addition, aerial peg infections and soil *A. flavus* populations were greater at 25/15ºC than35/25ºC. Infection and *A. flavus* population in the soil tended to increase with increasing $CO_2$ levels. However, the density of this fungus around the root and pod zones as observed by minirhizotron camera and analyzed by QuaCos was greater at 35/25ºC than at 25/15ºC. The incidence of *A. flavus* infection increased by 44-55% as [$CO_2$] increased from 400 to 800 µmol mol$^{-1}$. The fungal population density in the soil as indicated by relative green fluorescence color value after analyzing with QuaCos differed significantly among $CO_2$ concentrations but was not affected by temperature. The density of *A. flavus* population increased by 70-73% as $CO_2$ increased from 400 to 800 µmol mol$^{-1}$.

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In conclusion, high temperature (35/25°C) enhanced shoots and roots growth but reduced final reproductive biomass, which resulted from increased flower abortion and decreased seed size. There was no beneficial interaction of elevated $CO_2$ with higher temperature on the reproductive processes, despite the tendency for beneficial temperature by $CO_2$ interaction on vegetative growth and total shoot dry weight. At all levels of $CO_2$ concentration, higher temperature resulted in significant yield losses. The beneficial effects of $CO_2$ levels on photosynthesis and growth were overwhelmed by the negative effects of high temperature on reproductive growth. Thus, if climate change is associated with increased temperature, economic yield of crops that are sensitive to high temperature during the reproductive phase will be reduced even after taking account of the beneficial effects of $CO_2$ enrichment. Therefore, a global search for plant genotypes that are more tolerant to high temperature for seed production is needed for peanut and other seed crops to improve productivity at the present and in the future global climates.

In experiment 2, water deficit significantly reduced leaflet number, main stem length, total leaf area, SLA, shoot dry weight, and pod dry weight, but not leaflet area and branch number. Plants receiving T3 and T4 water treatments had shorter main stems, fewer leaflets, and smaller leaf area, SLA and shoot dry weight than T1 and T2 plants. Stem length reduction was greatest in Tainan 9, followed by 511CC and 419CC. The reduction of leaf area due to water deficit was greater in 419CC and Tainan 9 than 511CC. Shoot dry weight was greatest in 511CC. Root growth of peanut was influenced by soil moisture. Soil water potential at 72 cm depth for T1 and T2 plants remained near field capacity during the growth period, except at 83 DAP when soil water potential of T2 plants become slightly lower than field capacity (-0.03

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MPa). In T3 and T4 plants, soil water potential fell below field capacity at all soil depths. Genotype 511CC depleted soil water more than the other genotypes, with soil water potential at 5 cm soil depth reaching a minimum -0.9 MPa when received T3 water treatment and -1.4 MPa when received T4 water treatment, while Tainan 9 reduced soil water potential into -0.3 MPa and 419CC reduced into -0.2 MPa in T3 water treatment. In T4 water treatment, soil water potential of both genotypes was reduced to -1.0 MPa. Root length of plants receiving T1 and T2 water treatments tended to be longer at 5 and 25 cm. Whereas in T3 and T4 plants, root length was greater at 50 and 75 cm. Root length decreased with soil depth for plants receiving adequate water and tended to increase with soil depth for plant exposed to water deficit. Pod dry weight plant$^{-1}$ was severely reduced by water deficit, especially in 511CC for which the reduction was more than 60% in T3 plants and 80% in T4 plants. Pod weight reduction was from 32 to 57% in 419CC and from 28 to 53% in Tainan 9.

Water deficit increased *A. flavus* population density in the soil of all genotypes. The mean of green fluorescence value was greatest in T4 plants, followed by T3, T2, and least in T1 plants. Fungal colonization was relatively high in plants receiving T4 and T2 water treatments in both pod shell and seed. Recovery of *A. flavus* on pod shells increased with increasing water stress duration. Pod shells of all genotypes contained relatively high levels of *A. flavus* colonization (means 67-70%), whereas small seed infection was observed. Genotype 511CC, which is classified as aflatoxin resistant, appeared to have greater *A flavus* colonization in both pod shell and seed than the other two genotypes, even though it was found to have the lowest *A. flavus* population density in the soil.

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The study of biochemical responses of peanut pods to drought and *A. flavus* infection also showed that drought significantly increased the incidence of *A. flavus* infection. The greatest infection was found in 419CC, followed by Tainan 9 and 511CC. Drought altered the chemical compositional changes by reducing most of mineral compositions (except for Zn) and condensed tannin content of peanut pods, which may enhance susceptibility of peanut pod to fungal invasion. Crude protein increased but crude fat decreased when plants were subjected to drought. Genotype 511CC contained more condensed tannin, with known antifungal activity, in seed coat and also had lowest incidence of fungus colonization. These results suggest that tannin accumulation in seed coats may enhance in pre-harvest resistance to *A. flavus* infection and reduce subsequent aflatoxin contamination. Genotypes with high tannin content may be considered as at least partly-resistant. There were negative correlations among Mg and Mn with *A. flavus* infection. It appears that the Mg and Mn contents may either directly or indirectly be responsible for the susceptibility of peanut pod to *A. flavus* infection. Thus, the exact nature of nutrient disturbance, as well as whether or not the severity of *A. flavus* infection and subsequent aflatoxin contamination can be reduced by higher level of Mg or Mn in the plant remains to be investigated.

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In experiment 3, this experiment provides compelling evidence that preharvest peanut seed infection by *A. flavus* may occur systemically directly through floral infection. Initial infections may take place from different part of peanut flower organs as follow;

Infected pollen grain: Dense growth of mycelia of *A. flavus* on infected pollen grains was observed, which suggests that pollen plays a critical role in the

establishment of *A. flavus* growth on stigma and style. Pollen is a rich source of carbohydrates, amino acids, and minerals, which appears to be an excellent substrate for growth of this fungus. Pollen grains could be important in natural infection, because anthers form a relatively large area for fungal colonization and conidia produced on anthers are in a perfect position to act as secondary source of inoculum to infect other flower tissues.

Penetration through the stigma: As a saprophyte, *A. flavus* generally infects only injured tissues. I observed fungal conidia attached to the tips of stigma with pollen grain. Thus, when conidia germinated on the stigma surface and the fungal hyphae penetrated into the style following the same part of pollen tube, which effectively causes a channel of injury, infects the ovary and becomes established in developing pegs.

Penetration directly through the hypanthium, style, and ovary wall: This study, I observed the sporulation of *A. flavus* on hypanthium tissues within 48 hr after inoculation as well as occurring of fluorescent hyphae on the surface of an ovary at 24 hr after inoculation, and also inside an ovary tissue within 48 hr after inoculation. There is a possibility that *A. flavus* conidia may germinate on hypanthia and penetrate directly through this tissue and pass through the style until hyphae reach an ovary wall.

Observations of *A. flavus* colonization on plated, surface-sterilized terminal portions of aerial pegs developing from inoculated flowers after 10 days of inoculation confirm that *A. flavus* infection of peanut ovaries result from infection of floral tissues but this fungus may colonize peanut ovary non-pathogenically and remain associated with the apparently sound developing peanut peg tissues until stress occurs, such as drought, or until some other injury weakens tissues and allows *A.*

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*flavus* to infect additional tissues. The incidence of *A. flavus* colonization on aerial pegs was observed most thoroughly on Tainan 9, followed by 419CC and 511CC had the least. In conclusion, initial infection occurred anywhere on the surface of peanut flowers (stigma, style, pollen grain, hypanthium, standard, wing, and ovary). Pollen grain, stigma, and style appeared to be particularly susceptible to colonization as fungal hyphae were often found in these tissues. In recognition that floral infection is likely to be the most important pathway of infection, thus knowledge of the floral infection could be a key to optimizing control of preharvest *A. flavus* infection and subsequent aflatoxin contamination, and then research would be warranted to identify irrigation, row orientation and other factors that would prevent the movement of conidia form the soil surface to the flowers.

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