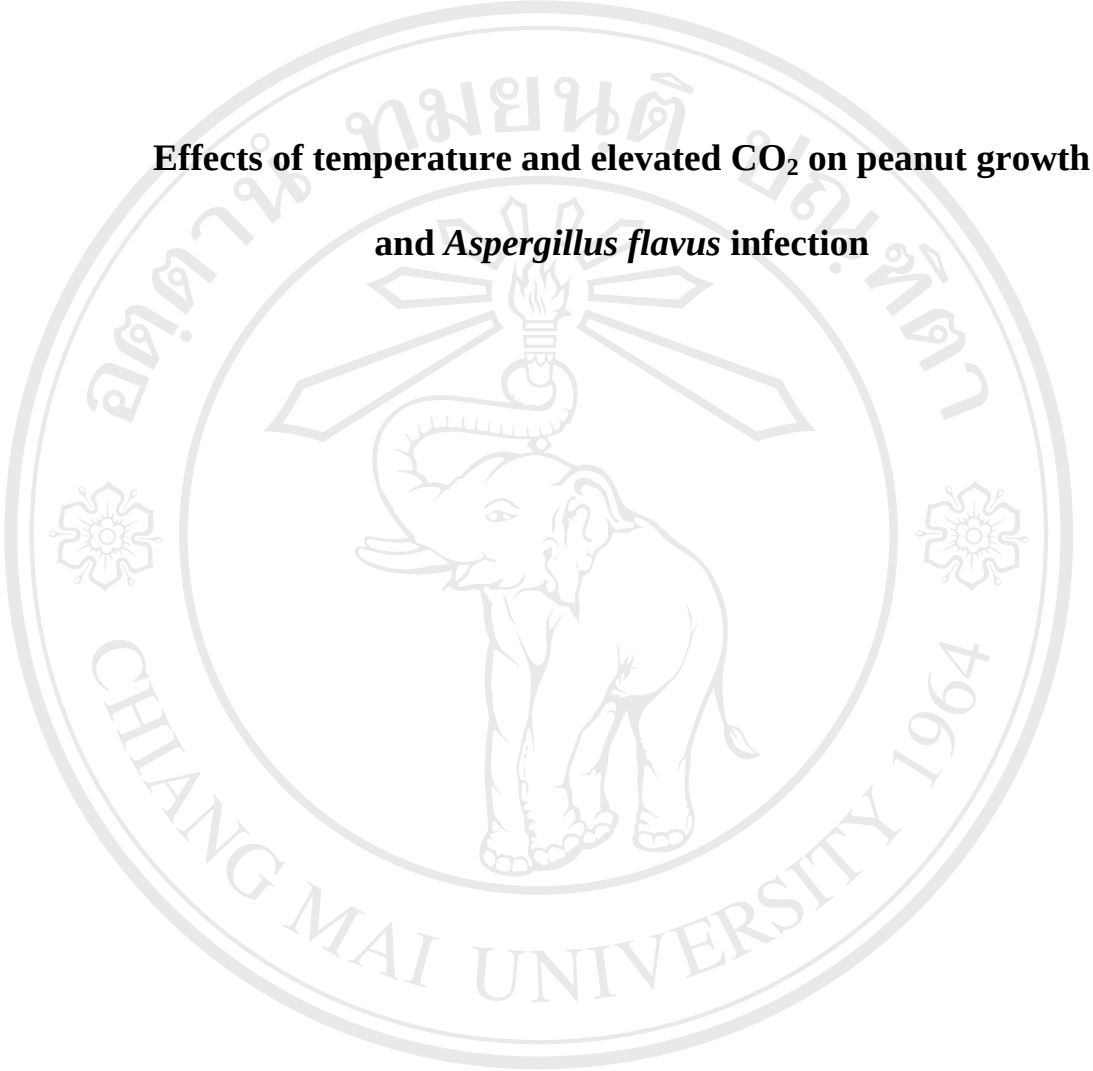
EXPERIMENT 1

Effects of temperature and elevated CO_2 on peanut growth and *Aspergillus flavus* infection



INTRODUCTION

Global climate change has emerged as an important environmental challenge due to its potential impact on biological systems on Earth (Walther *et al.*, 2002). There is considerable concern about the increasing carbon dioxide concentration ($[CO_2]$) in the atmosphere, associated increases in temperature and their effects on crop production. At the present rate of emission, atmospheric $[CO_2]$ is projected to be in the range of 540 to 970 μmol mol^{-1} by the year 2100. Rising concentrations of carbon dioxide and other greenhouse gases, including methane and nitrous oxide, will potentially increase global average near surface temperatures by 1.4 to 5.8ºC (Houghton *et al.*, 2001). Therefore, it is important to quantify the interactive effects of increasing $[CO_2]$ and temperature on crop production.

Peanut (*Arachis hypogaea* L.) is an important oil seed crop which is grown as a principle source of edible oil and vegetable protein. About 90% of the world's peanut is produced in tropical and semi-arid tropical regions, which are characterized by high temperatures and erratic rainfall. In the tropics, most crops are near their maximum temperature tolerance; therefore, crop yields would likely decrease with minimal increases in temperature. The mean optimal air temperature range for vegetative growth of peanut is from 25 to 30ºC, which is warmer than the optimum range for reproductive growth, which is from 22 to 24ºC (Cox, 1979; Del Castillo *et al.*, 1989). Both short- and long-term exposure to air and soil temperatures above the optimum can cause significant yield loss in peanut (Golombek and Johansen, 1997; Prasad *et al.*, 2000a). Day temperatures greater than 34ºC decreased fruit-set and resulted in fewer pods (Prasad *et al.*, 1999, 2000a). Decreased fruit-set at high

temperatures was mainly due to poor pollen viability, reduced pollen production, and poor pollen tube growth, all of which lead to poor fertilization of flowers.

The increase in photosynthetic rate and pod yield of peanut due to elevated [CO_2] have been reported by many researchers (Chen and Sung, 1990; Stanciel *et al.*, 2000). Net photosynthesis increased linearly for some peanut genotypes as [CO_2] increased from 300 to 600 μmol mol^{-1}. Net photosynthetic rate of peanut grown under controlled environmental conditions was highest among plants grown at 800 μmol mol^{-1}. The CO_2 enrichment from 400 to 800 μmol mol^{-1} had positive effects on peanut growth and yield, but increasing [CO_2] to levels greater than 800 μmol mol^{-1} led to only marginal seed yield increases (Stanciel *et al.*, 2000). High [CO_2] increased biomass and pod yields of Virginia-type peanut (Chen and Sung, 1990).

Although, analyses of plant responses to elevated atmospheric [CO_2] have focused largely on the processes occurring above ground, an understanding of photosynthesis alone is not sufficient to answer the important questions about terrestrial responses to a changing atmosphere. A whole-plant perspective is needed to understand the critical feedbacks and adjustments that occur within a plant and between plant and soil. Thus, an understanding of root system responses is also important. There are fewer belowground than aboveground investigations in the CO_2 response literature. The most commonly reported on root variable is root dry weight (Rogers et al., 1994), which has been reported to increase with elevated [CO_2] for such crops as sorghum (Chaudhuri *et al.*, 1986), soybean (Rogers *et al.*, 1992a), and winter wheat (Chaudhuri *et al.*, 1990). These results suggest that root systems more thoroughly exploit a given volume of soil under elevated [CO_2]. Roots of CO_2 enriched plants reach deeper soil layers (Rogers *et al.*, 1992a) or attain maximum

rooting depth ahead of plants grown under ambient [CO_2] (Chaudhuri *et al.*, 1990). High [CO_2] increased stele and cortex diameters, root diameter in the root hair zone, length of unbranched first order lateral roots, and total root length and volume of cotton (Rogers *et al.*, 1992b).

Moreover, carbon dioxide-induced changes in plants will affect the structure and function of rhizosphere and soil microorganisms. As pathogens, symbionts, and decomposers, microbes exert a strong influence on carbon and nutrient cycling in plant/soil systems. Change in plant structure (Prior *et al.*, 1995), physiology (Roger *et al.*, 1994), and phytochemistry (Prichard *et al.*, 1997) brought about by elevated atmospheric CO_2 may alter plant-microbe interaction in the soil. In addition, root exudation, which can increase in plants grown under CO_2 enrichment (Norby *et al.*, 1987), might be provided as a substrate for *A. flavus* growth in the soil, which may increase peanut pod infection by this fungus.

Under future climate change scenarios, it is likely that plants will be exposed to a combination of both higher temperature and [CO_2] (Rosenzweig and Hillel, 1998). Therefore, it is important to understand the combined effects of elevated [CO_2] and temperatures for determining crop management or genetic improvement required to sustain peanut productivity in a future climates. Little work has been done to study the combined effects of elevated [CO_2] and temperature on peanut growth and also *A. flavus* infection. Thus, the objective of this study were (i) to determine the growth and development responses of peanut shoot and root grown under different air temperatures and atmospheric [CO_2] combinations and (ii) to evaluate the effect of these treatment combinations on *A. flavus* growth and infection.

MATERIALS AND METHODS

The research was carried out in the six controlled environment growth chambers at the Georgia Envirotron, The University of Georgia, Griffin, Georgia, USA during August to December 2002. This research was divided into two sub-experiments, comprised of evaluating shoot and root growth and *A. flavus* infection of peanut that growing in 20-L container and assessing root growth and distribution in the thin-layer soil rhizotron system.

Sub-experiment 1: Effects of temperature and elevated CO_2 on peanut growth and *Aspergillus flavus* infection

Plant material and growth conditions

Healthy and uniform seeds of Tainan 9 peanut variety (*Arachis hypogaea* L.) were pre-germinated by wrapping them in moist paper and placed in a controlled environment growth chamber (GC72, Conviron, Winnipeg, Manitoba, Canada) with 30°C day/25°C night temperature for five days. Two germinated seeds were grown in plastic 20-L containers filled with loamy sand soil. Each container was fitted with a minirhizotron tube at 5 cm below the soil surface. Containers were arranged in a factorial completely randomized design with six replications. Six containers were placed in each controlled environment chambers (Figure 2.1). Chambers were set to six treatment combinations: (1) 25/15°C day/night air temperature and 400 μmol mol^{-1} atmospheric CO_2 concentration [CO_2]; (2) 25/15°C and 600 μmol mol^{-1} [CO_2]; (3) 25/15°C and 800 μmol mol^{-1} [CO_2]; (4) 35/25°C and 400 μmol mol^{-1} [CO_2]; (5) 35/25°C and 600 μmol mol^{-1} [CO_2]; and (6) 35/25°C and 800 μmol mol^{-1} [CO_2].

Throughout the growing period, day/night relative humidity was maintained at 75/95%, maximum light intensity was 1500 μmol PAR $m^{-2}s^{-1}$, and photo period was 16 h.

In the 25/15°C temperature treatments, plants were irrigated with tap water twice weekly until drainage occurred, while those in 35/25°C treatments were watered daily at an amount sufficient to avoid water deficit. A modified half-strength Hoagland's nutrient solution, as described in Table 2.1 (Hoagland and Arnon, 1950), was applied to the plants weekly to assure adequate nutrient for plant growth and development. Plants were kept weed free and healthy throughout the experiment by hand weeding. Insecticides were sprayed to prevent incidence of aphids (*Aphis fabae* Scopoli), white fly (*Bemisia tabaci* Gennadius), and red spider mites (*Tetranychus urticae* Koch).

Figure 2.1 Two peanut plants growing in 20-L containers, each fitted with a minirhizotron observation tube at 5 cm soil depth.

Inoculum preparation and application

Spore suspension

Inoculations used two strains of *Aspergillus flavus* that were modified to produce a green fluorescent protein (GFP) with yellow green-fluorescence when exposed to UV light (Appendix A). One strain was produced by Gary Payne (NCSU, Releigh, North Carolina), the other by Jeffrey Carey (USDA-ARS, New Orleans, Louisiana). The two *A. flavus* strains were maintained on M3S1B, a selective medium for *A. flavus* and *A. niger* groups (Griffin and Garren, 1974). This medium consisted of 5.0 g peptone, 10.0 g glucose, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 30.0 NaCl, 20.0 g agar, 50.0 mg streptomycin sulfate, 50.0 mg chlorotetracycline, 1.0 mg 2, 6-dichloro-4-nitroaniline (added in 3 ml acetone), and 1 liter distilled water. The *A. flavus* cultures were incubated at 30°C for 7 days. Then conidia were washed from the Petri dishes using sterile distilled water containing 10 drops L^{-1} of Tween 20 and filtered through three layers of sterile cheesecloth. The concentration of conidia was determined with a hemacytometer and adjusted with sterile distilled water to 4×10^6 conidia ml^{-1}. Inoculum was refrigerated at 4°C until used.

Cracked corn inoculum

Cracked corn inoculum was prepared by placing 200 g of cracked corn in a 500 ml flask, then autoclaving twice. After cooling, moisture content of cracked corn in the flask was adjusted to 25% by adding 50 ml spore suspension of *A. flavus*. The flask was sealed and incubated at 30°C for 7 days. The flask was shaken daily to provide for uniform growth and distribution of the fungus throughout the medium. The cracked corn inoculum was stored in a refrigerator until used.

At 23 DAP, 25 g of cracked corn inoculum was incorporated into the top 1-cm soil layer of each container, thereafter a hand pump spray with spore suspension was sprayed over the shoots and flowers at 53 and 73 DAP.

Ten aerial pegs of each treatment were collected at 85 DAP to evaluate for *A. flavus* infestation. Pegs were surfaced sterilized by soaking in 10% Clorox solution for 180 s. Samples were then rinsed twice with sterile water and excess water was removed by placing samples in sterile paper towels to help reduce bacterial growth. Pegs were dissected longitudinally and plated on Petri dishes with M3S1B medium. Cultures were incubated at 30^{o}C for 5 days at which time the percent of pegs infected with *A. flavus* was recorded.

At 99 DAP, a minirhizotron camera (Appendix B) with built in UV lighting system (BTC100-X, Bartz Technology Corp., Santa Barbara, CA 93101, U.S.A.) was used to observe GFP *A. flavus* populations on surface of roots, pegs, and pods at 5 cm depth by intensity of fluorescence when illuminated with UV light. Digital images were directly recorded 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 pixel or group of pixels in a digital image and produces a spreadsheet of values for color intensities. Only green color value was analyzed because of GFP *A. flavus* fluoresces green. Green color intensity was scored on a 0 to 255 scale. Full images were analyzed in 10 × 10 pixel groups. Total number of group was analyzed 64 × 48 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).

Soil was collected from each container at 99 DAP. Soil samples were air dried for 24 h and sieved trough 2 mm mesh screen. Then, a 10-g sample of soil was suspended in 90 ml sterile water in 200-ml bottle. The suspension was thoroughly mixed by shaking for 15 min. A sterile pipette was used to transfer 1 ml of each suspension to a tube containing 9 ml of sterile water. Then, a fresh sterile pipette was used to mix and transfer 1 ml of this suspension to a second tube containing 9 ml of sterile water. This process continued until the number of dilutions reached 10^{-4} and 10^{-5} concentrations. For the final dilution, 0.5 ml of suspension was taken by a sterile micropipette and transferred to each of two sterile Petri dishes containing M3S1B medium and carefully spread over surface agar. The plates were incubated at 30^{o}C for 3 days, and then fungal colonies were counted. Populations of *A. flavus* in soil sample were recorded as colony-forming units g^{-1} soil.

Crop growth measurements

The time from planting to first flower of each treatments was recorded. Shoots traits, including main stem length and number of branches, were observed weekly from 43 DAP onward. The youngest fully expanded leaf on the main stem was detached for leaf area (LA), dry weight, and specific leaf area (SLA) determination at 14-day intervals.

A minirhizotron camera was used to observe roots at the top of minirhizotron tube. Digital root images were collected at 14-day intervals. Images were analyzed using RMS software (Ingram and Leers, 2001). Data on root length, diameter, and number were obtained for each image taken.

Shoots, roots, and pods were harvested by hand at 112 DAP. Fifty leaves in each container were separated and leaf area was measured using a leaf area meter (Model Li-3000, Li-Cor, Inc., Lincoln, NE). Root samples were taken by submerging the soil container upside down in the water. Roots were carefully washed from the soil. Fresh shoots and roots were dried at 70^{o}C for 72 hr and then dry weights were recorded. Pods were removed from each plant by hand immediately after shoot harvesting. Harvested pods were cleaned by tap water, air dried for 7 days, and then weighed. Plants grew from 12 August 2002 and final sampling was done on 2 December 2002 for a total growth period of 112 days.

Table 2.1 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	$MnSO_4.H_2O$	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 each stock A and stock B in 1 liter of water.

Sub-experiment 2: Peanut root growth responses to different atmospheric temperature and CO_2 concentration

Rhizotrons (Figure 2.2 A) were made of transparent acrylic material with a front and a back plate, leaving a soil layer of 6 mm thickness after closing the two compartments and compression of the soil layer (500 mm length × 450 mm width × 6 mm height), as described by Kuchenbuch and Ingram (2002). A loamy sand soil was used in this research. Soil was sieved twice through a 1 mm × 1 mm screen, and placed in the well of the front plate. The soil was spread evenly to give a uniform layer about 10 mm thick. The back plate was mounted and the soil layer was compressed to 6 mm thickness using a hydraulic press (Model 25 H, Dake Corp., Grand Haven, MT). Front and back plates were protected against deformation by a support system covering the entire surface and bolted firmly together while still under pressure. Soil bulk density in each rhizotron was 1.22 g cm^{-3}.

Plant material and growing conditions

Two germinated seeds of peanut cultivar Tainan 9 were planted in each rhizotron when the radicle was 1 to 3 mm long then covered with 10 mm soil. Rhizotrons with plants were placed in 6 different controlled environment chambers on the racks slanted to a 45^{o} angle with the front plate facing downward to promote root growth along the front plate and covered with opaque black plastic film to prevent light from entering rhizotrons between observations. Chambers were set to six treatment combinations of two temperatures (25/15 and 35/25ºC, day/night temperature) and three levels of atmospheric [CO_2] (400, 600, and 800 µmol mol^{-1}). Throughout the growing period, day/night relative humidity was maintained at

70/95%, maximum light intensity was 1500 µmol PAR $m^{-2}s^{-1}$, and photo period was 16 h. After planting, 360 ml of water was added to the top of each rhizotron and watered every other day thereafter until the experiment ended.

Scanning procedure

At 3, 7, 10, 14, and 17 days after planting, the front part of each rhizotron was placed on flatbed scanner (Model MRS-1200A3, Microtek International, Inc, Taiwan, ROC) to collected digital images of visible roots. Each rhizotron required two images from the scanner, one for the top and another for the bottom half of the rhizotron. The total image area of these initial images was 350 mm wide × 250 mm high. Optical resolution used was 300 dpi, with 256 colors. Images were cropped to 175 mm wide × 100 mm high, giving 10 images for each rhizotron. Diameter and length of roots visible on the images collected with the scanner were evaluated using RMS software. In short, an operator used a computer mouse to trace each visible root while adjusting the circular cursor to match the diameter of the root. At the end of each image, RMS computes and stores measured values of root length and diameter for each segment, and total number of roots. Root length is stored both as a total and within root diameter classes.

Measuring total root length in the soil

At 17 days after planting, the back plate of each rhizotron was removed and a soil strip of 25 mm along the sides and bottom was removed to avoid edge effects. The remaining soil was subdivided in 10 areas, each 175 mm × 100 mm (Figure 2.2 B), thereby matching the size and location of images taken with the scanner. Soil samples were stored at 5ºC for at most 5 days before roots were carefully washed

from soil samples using a sieve with 1 mm mesh size and a low-pressure nozzle. Cleaned roots were placed in ethyl alcohol (25% v/v), to which methyl violet dye had been added. Roots were refrigerated and stored for at least 3 days to assure even coloration of roots. Total root length was analyzed using WinRhizo software (Regent Instruments, Inc., Quebec, Canada).

Statistical analysis

Statistical analyses were performed using SAS statistical package, Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA.). The analysis of variance (ANOVA) was used to determine the effects of temperature, CO_2, and their interactions on measured variables. Means were compared by least significant difference (LSD).

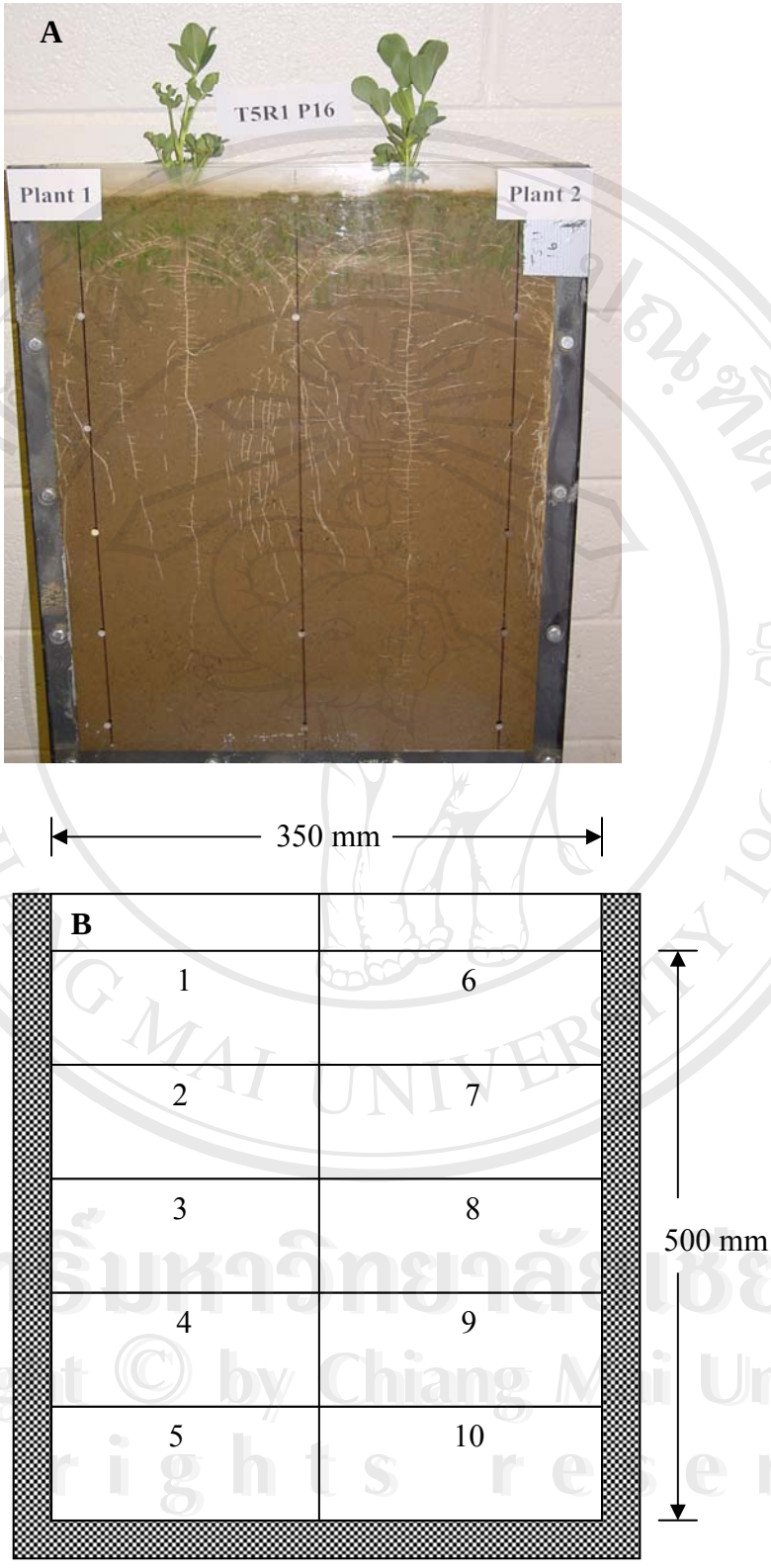


Figure 2.2 Two peanut plants growing in a rhizotron (A) and schematic representation of subdivisions of transparent surface for image acquisition (B).

RESULTS

Effects of temperature and elevated CO_2 on peanut growth and *Aspergillus flavus* infection (Long-term experiment)

Time from planting to first flower differed between temperature treatments, but there was no effect of CO_2 levels. Duration from planting to first flower at day/night temperature of 25/15ºC was 34 days and at 35/25ºC was 22 days, for both ambient and elevated [CO_2].

Figure 2.3 shows the effects of temperature, CO_2, and their interactions on main stem length. Main stem lengths increased linearly with increasing [CO_2] from 43 to 99 DAP in both low and high temperature treatments. Main stems of plants grown at 35/25ºC were longer than those at 25/15°C for all [CO_2] from 43 through 71 DAP, after which main stems of plants in the 25/15°C, 800 µmol $mol^{-1}CO_2$ treatment were longer than those at high temperature. At 25/15ºC, plants grown at 600 and 800 µmol mol^{-1} CO_2 had main stems that were 24 and 44 % longer than those plants grown at 400 µmol mol^{-1} CO_2, while at 35/25ºC the main stem was similar with about 37.8 cm in all [CO_2]. There were no significant effects of [CO_2] or temperature on number of branches per plant; therefore branch number of all treatment combinations was approximately 12 $plant^{-1}$.

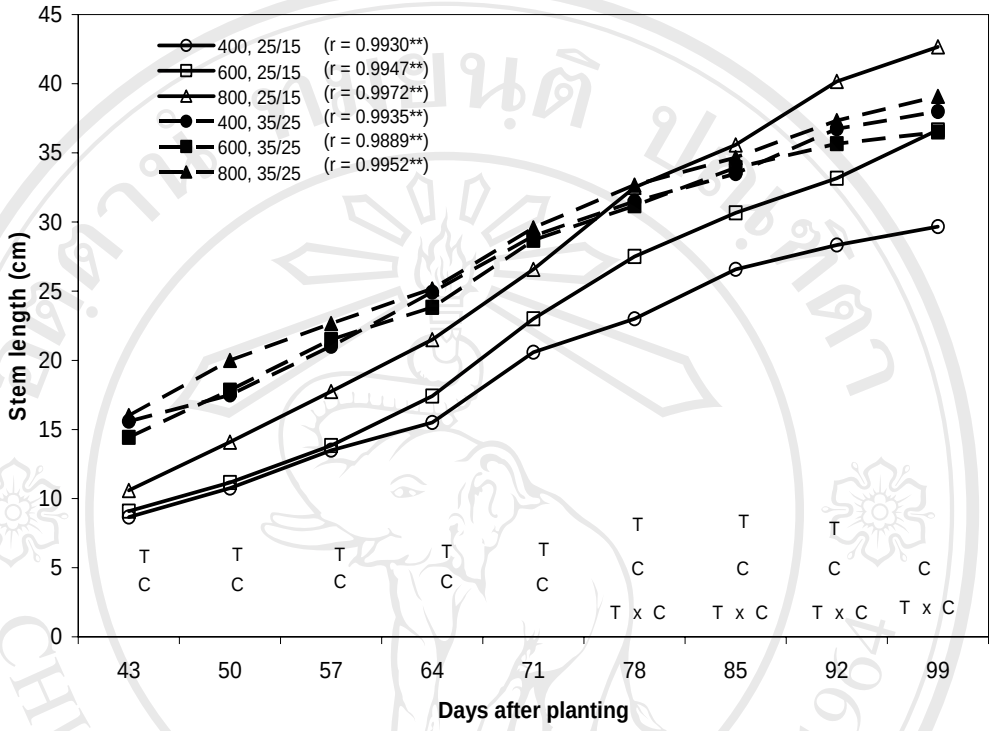


Figure 2.3 Effect of temperatures and CO_2 concentrations on main stem length at different plant ages. Letters above each plant age indicate significant differences ($P \leq 0.05$) between 25/15ºC and 35/25ºC temperature treatments, T; between CO_2 levels (400, 600, 800 μmol mol^{-1}), C; and interaction between temperature and CO_2, T × C.

There was no significant effect of [CO_2] on individual leaf area at any time of observation (Figure 2.4 a). Plants at 25/15ºC had larger individual leaf area than those at 35/25ºC. At 25/15ºC, leaf size increased rapidly from 43 to 71 DAP and slightly increased thereafter for plants exposed to 800 µmol mol^{-1}, but declined from 85 to 99 DAP at 600 µmol mol^{-1} and from 71 to 99 DAP at 400 µmol mol^{-1} CO_2. At 35/25ºC, leaf sizes of plants growing at 400 µmol mol^{-1} increased progressively from 43 to 99 DAP while those growing at 600 and 800 µmol mol^{-1} CO_2, leaf size increased until 71 DAP, declined at 85 DAP, then increased thereafter.

Figure 2.4 b shows the dry weight of individual leaves. Leaf dry weights of plants growing at low temperature were greater than those at high temperature. At 25/15ºC, the maximum leaf dry weight was observed at 71 DAP, and ranged from 0.47 to 0.54 g $leaf^{-1}$as [CO_2] increased from 400 to 800 µmol mol^{-1}. At 35/25ºC, the maximum leaf dry weight was attained at 99 DAP, and ranged from 0.19 to 0.22 g $leaf^{-1}$as [CO_2] increased.

At 112 DAP there were significant effects of CO_2 and temperature on total leaf area and specific leaf area (Table 2.2). Increasing temperature from 25/15ºC to 35/25ºC significantly reduced total plant leaf area under all levels of [CO_2]. Total leaf area per plant was about 921.2 cm^2 for 25/15ºC and 573.7 cm^2 for 35/25ºC. It was greatest when plants received 600 µmol mol^{-1} CO_2 with the average of 828 cm^2, followed by at 800 µmol mol^{-1} CO_2 with the average of 725 cm^2 and at 400 µmol mol^{-1} CO_2 with the average of 689 cm^2. Specific leaf area of plants grown at 35/25ºC was greater than those at low temperature. Specific leaf area was about 186 cm^2 g^{-1} for 400 µmol mol^{-1} CO_2, 174 cm^2 g^{-1} for 600 µmol mol^{-1} CO_2, and 193 cm^2 g^{-1} for 800 µmol mol^{-1} CO_2.

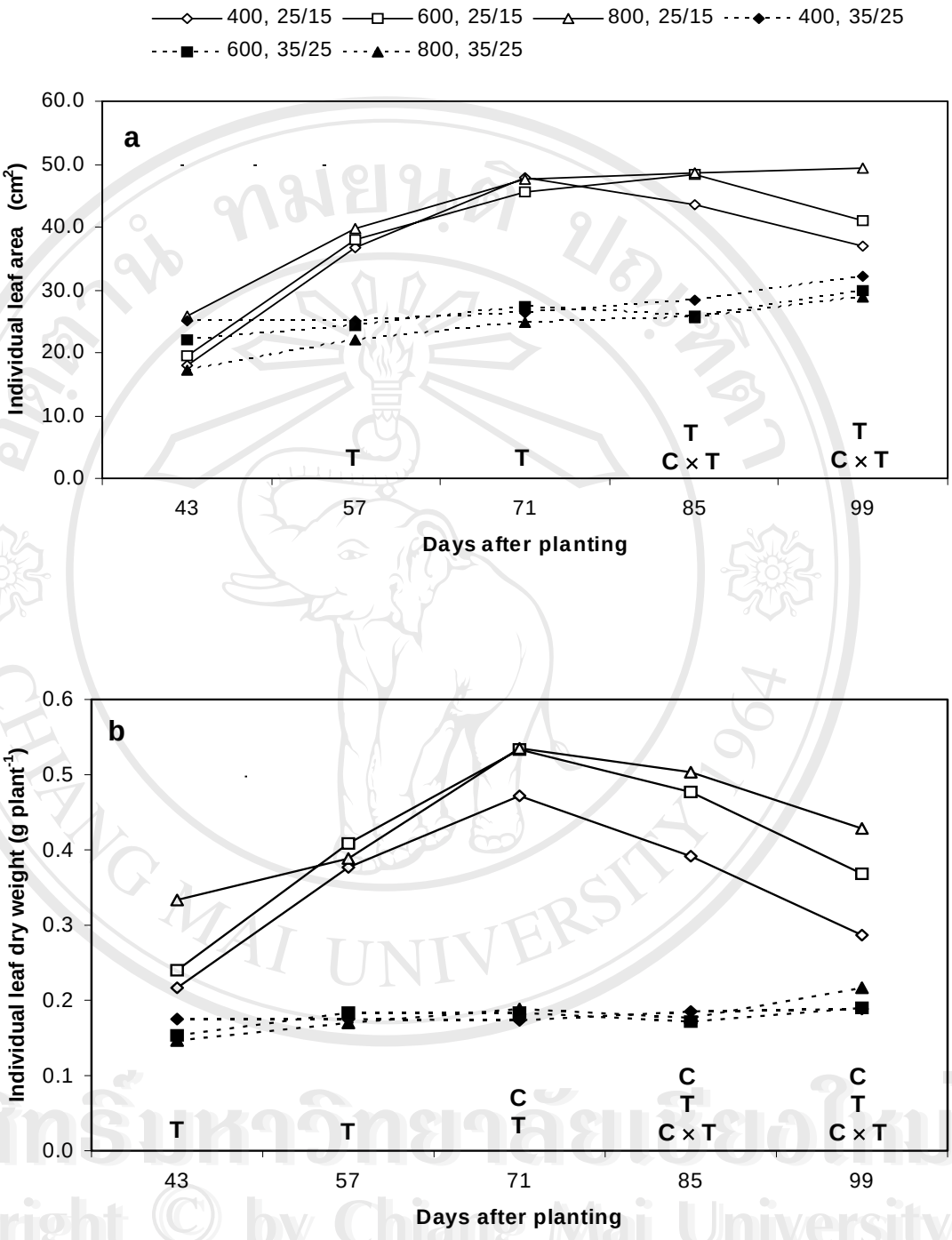


Figure 2.4 Effect of temperature and [CO_2] of on individual leaf area (a) and leaf dry weight (b) at different plant ages. Letters above each plant age indicate significant differences ($P \leq 0.05$) between 25/15ºC and 35/25ºC temperature treatments, T; between CO_2 levels (400, 600, 800 μmol mol^{-1}), C; and interaction between temperature and CO_2, T × C.

Above ground biomass increased with increasing CO_2 from 400 to 800 µmol mol^{-1} in plants grown at 25/15ºC, whereas at 35/25ºC above ground biomass was greater at 800 than 400 µmol mol^{-1} CO_2 but slightly declined at 600 µmol mol^{-1}. The above ground biomass increased by 56% at 400µmol mol^{-1}, 24% at 600 µmol mol^{-1}, and 16% at 800 µmol mol^{-1} CO_2 as temperature increased from 25/15ºC to 35/25ºC. It was greatest when plants were grown under 35/25ºC and 800 µmol mol^{-1} CO_2 (Table 2.2).

Elevated [CO_2] increased total pod dry weight when plants were grown under 25/15ºC, but not in the 35/25ºC treatment (Figure 2.5). In the 25/15ºC treatment, pod dry weight was 50% greater than in the 35/25ºC treatment. As temperature increased from 25/15ºC to 35/25ºC, pod dry weight was reduced by 40% at 400, 53% at 600, and 54% at 800 µmol mol^{-1}.

Fibrous root dry weight increased as [CO_2] increased when plants were grown at 25/15ºC treatments, but decreased with increasing [CO_2] at 35/25ºC treatments. At 35/25ºC, plants had greater root dry weight about 34% at 400, 14% at 600, and 7% at 800 µmol mol^{-1} compared with 25/15ºC treatment (Table 2.2).

Fibrous root dry weight increased as [CO_2] increased when plants were grown at 25/15ºC treatments but decreased with increasing [CO_2] at 35/25ºC treatments. At 35/25ºC, plants had greater root dry weight about 34% at 400, 14% at 600, and 7% at 800 µmol mol^{-1} compared with 25/15ºC treatment (Table 2.2).

Minirhizotron observations showed significant effects of temperature at 43, 57, 85, and 99 DAP for total visible root length and number of roots, and at 57, 85, and 99 DAP for root length density; RLD (data of root number and RLD are not shown). Visible root length, number of roots, and RLD in the minirhizotron tubes were

significantly greater for plants grown under 35/25ºC than for those grown under 25/15ºC at all CO_2 levels. At 99 DAP, plants grown under high temperature had 23% greater visible root length, 37% greater root number, and 23% greater RLD than those at 25/15ºC. There was no effect of elevated [CO_2] on those observed parameters during the course of observation. However, visible root length, root number, and RLD tended to increase with increasing [CO_2] from 400 to 800 μmol mol^{-1} at 25/15ºC, but decreased as [CO_2] increased at 35/25ºC (Figure 2.6).

Table 2.2 Vegetative growth and total biomass at 112 days after planting of peanut grown in three atmospheric CO_2 levels and two air temperature treatments.

CO_2 (μ mol mol^{-1})	Main stem length			Branch number			Leaf area		
	25/15°C	35/25°C	Mean	25/15°C	35/25°C	Mean	25/15°C	35/25°C	Mean
	----------cm----------						---------cm^2 $plant^{-1}$---------		
400	30	38	34	12	11	12	805	574	690
600	37	37	37	13	11	12	1052	605	829
800	43	39	41	13	13	13	908	543	723
Mean	37	38	38	13	12	13	922	574	748
LSD (T)*		n.s			n.s			79.5	
LSD (C)†		2.9			n.s			97.4	
LSD (TxC) ‡		4.1			n.s			n.s	
CO_2 (μ mol mol^{-1})	Specific leaf area			Root dry weight			Shoot dry weight		
	25/15°C	35/25°C	Mean	25/15°C	35/25°C	Mean	25/15°C	35/25°C	Mean
	--------- cm^2 g^{-1}----------			---------g $plant^{-1}$---------			---------g $plant^{-1}$---------		
400	170	203	187	57	76	67	203	317	260
600	155	193	174	63	72	68	239	296	268
800	175	212	194	65	69	67	289	336	313
Mean	167	203	185	62	72	67	244	316	280
LSD (T)*		9.4			n.s			12.9	
LSD (C)†		11.5			n.s			15.8	
LSD (TxC) ‡		n.s			n.s			22.3	

* Least significant difference (LSD) at $P \leq 0.05$ for comparing means among the two air temperature treatments at CO_2 concentration of 400, 600, and 800 (μmol mol^{-1}).

† Least significant difference (LSD) at $P \leq 0.05$ for comparing means between the three atmospheric CO_2 concentrations at air temperature treatment of 25/15 and 35/25ºC.

‡ Least significant difference (LSD) at $P \leq 0.05$ for temperature by CO_2 interaction.

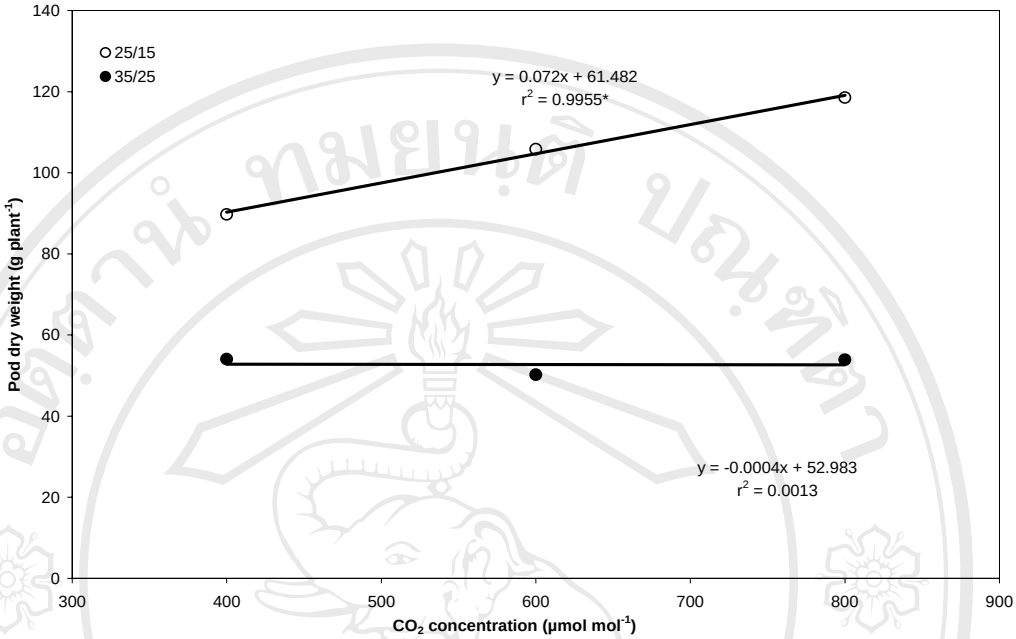


Figure 2.5 Relation between CO_2 concentration and pod dry weight per plant of peanut at 25/15ºC and 35/25ºC.

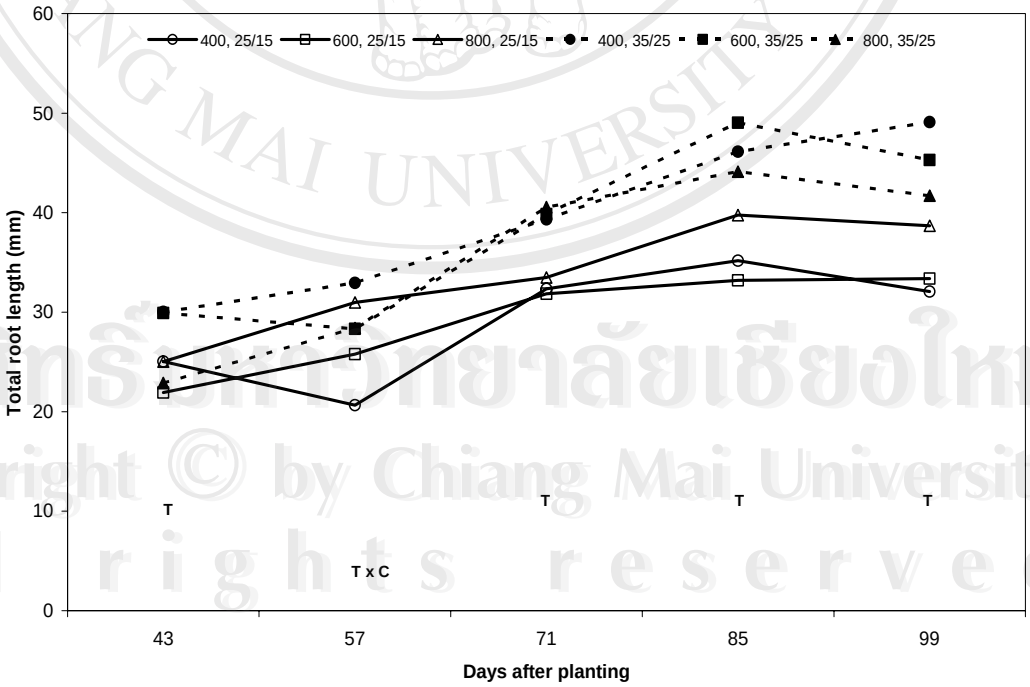


Figure 2.6 Total root length at different temperature regimes and CO_2 concentrations as observed by minirhizotron camera with increasing days after planting. Letters indicated significant effects of temperature (T), CO_2 (C), and interaction between temperature and CO_2 (T × C), ($P \leq 0.05$).

Infection and colonization of peanut by *A. flavus*

Aerial peg infection by *A. flavus* was determined at 85 DAP. The sporulation of this fungus on aerial peg tissue plating on medium culture was observed within 5 days after incubation. There was no statistically significant effect of temperature or CO_2 concentration on aerial peg infection by *A. flavus*. However, percent of fugal colonization of plant exposed to 25/15ºC temperature was greater than at 35/25ºC in all overall [CO_2] (Figure 2.7 a). The incidence of infection increased with increasing CO_2 level. The percent of infection were 55%, 48% and 44% for plant receiving 800, 600 and 400 µmol $mol^{-1}CO_2$, respectively.

The density of *A. flavus* population in the soil as evaluated by soil serial dilution method at 99 DAP was shown in Figure 2.7 b. Soil population of plant grown under 25/15ºC temperature was significantly greater than those grown at 35/25ºC. The population of *A. flavus* was decreased by 58% when temperature increased. Among the three CO_2 concentrations, the density of *A. flavus* population was greatest in 800 µmol mol^{-1}(7.1×10^5 cfu/ g soil), followed by in 600 µmol mol^{-1}(3.7×10^5 cfu/ g soil) and in 400 µmol mol^{-1} (2.1×10^5 cfu/ g soil). However, the statistically significant different was not found between CO_2 level.

A minirhizotron camera with built in UV lighting system allowed me to observe *A. flavus* growth on the surfaces of root and pod zone at the 5 cm soil depth. The higher fluorescence was mostly observed in the pod zone as compared to the root zone. The data of *A. flavus* population density in the soil as indicated by relative green fluorescence color value after analyzing with QuaCos is presented in Table 2.3. The fungal population differed significantly between [CO_2] but not in temperature. The highest *A. flavus* density in the soil was found in plant receiving 800 µmol $mol^{-1}CO_2$.

The density of *A. flavus* population increased by 70-73% with CO_2 increased from 400 to 800 μmol mol^{-1}. Among two temperature regimes, the density of fungus population in the soil was larger in the plants exposed to 35/25ºC than those growing at 25/15ºC. However, there were not statistically significant differences.

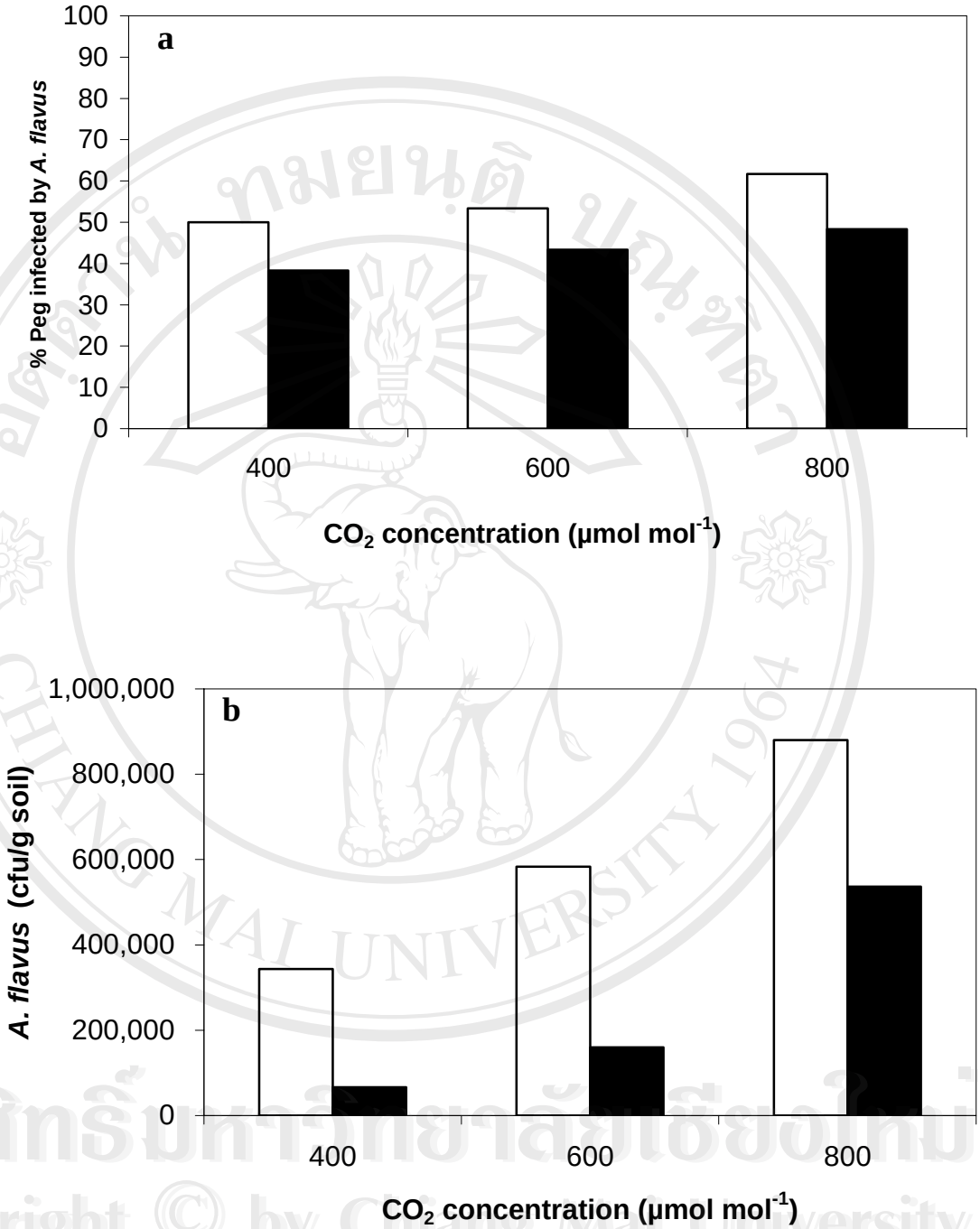


Figure 2.7 Effect of temperatures and CO_2 concentrations on aerial peg infection by *Aspergillus flavus* observed at 85 DAP (a) and *A. flavus* population in the soil at 85 days after planting (b).□= 25/15°C, ■= 35/25°C.

Table 2.3 Relative *Aspergillus flavus* population density as estimated by green fluorescence color value (64 × 48 groups of each image) in the root zone at 5 cm soil depth at harvest of Tainan 9 peanut grown under 6 different CO_2 by temperature combinations in the growth chambers of the Georgia Envirotron.

CO_2 ($\mu mol\ mol^{-1}$)	Temperature (day/night, °C)		Mean
	25/15	35/25	
	Relative green fluorescence color value, image total		
400	3,219	4,125	3,672 c
600	12,258	12,021	12,140 b
800	13140	13,941	13,540 a*
Mean	9,539 ns	10,029 ns	9,784

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

Peanut root growth responses to different atmospheric temperature and CO_2 concentration (Short-term experiment)

For all treatments in the rhizotron experiment, primary roots penetrated the soil nearly vertically relative to the rhizotron and most first order laterals root grew nearly horizontal. Tap roots reached 500 mm soil depth within 13 to 14 DAP for plants growing in the high temperature regime and within 16 to 17 DAP for those in the low temperature regime. The number of first order branch roots generated from main root axis was 11% greater for plants growing at high than at low temperature (Figure 2.8).

Figure 2.9 shows non-destructive root length measurements using RMS to analyze images collected with a scanner on 3, 7, 10, 14, and 17 DAP. Total visible root length of plants growing under low temperature increased exponentially until 10 DAP, and increased linearly thereafter, while visible root length of those growing under high temperature increased rapidly from 3 to 10 DAP, and 14 to 17 DAP. Except for during 10 to 14 DAP, root length slightly increased. Low temperature significantly increased number of roots per plant, at 3, 14, and 17 DAP, while the effect of elevated [CO_2] was significant only at 10 DAP. Plants in the low temperature treatments had more roots than those in the high temperature treatments. Root number was greatest at 600 μmol mol^{-1} CO_2.

Figure 2.10 shows visible root length in the five 100-mm soil layers of the rhizotron as measured by RMS at the end of the study (17 DAP). Visible root lengths under 25/15ºC were greater in the three upper layers (i.e., 0-100 mm, 100-200 mm, and 200-300) mm whereas at 35/25ºC, visible root length was greater only at the two upper layers (i.e., 0-100 mm and 100-200 mm). Temperature significantly affected

visible root length in the 200-300 mm and 300-400 mm soil layers. Plants grown in the 25/15ºC regime had 47% greater visible root length in the 200-300 mm soil layer and 62% greater root length in the 300-400 mm soil layer than those at 35/25ºC.

Temperature effects on root numbers were significant in the upper four soil layers, but not at 400-500 mm. Compared with plants at 35/25ºC, number of roots for plants at 25/15ºC were 23% greater in the 0-100 mm layer, 40% in the 100-200 mm layer, 47% greater in the 200-300 mm layer, and 41% greater in the 300-400 mm soil layer. There was no significant effect of [CO_2] on root numbers.

Figure 2.11 compares root length visible at the transparent rhizotron surface as measured by RMS with roots washed from the entire soil as measured by WinRhizo. The visible root length was 26% to 33% of the total root length present in the soil. The coefficient of determination, $r^2 = 0.96$ indicates that it is possible to estimate total root length from root measurements conducted at the transparent surface. There were significant effects of temperature on both total root length (WinRhizo) and visible root length (RMS). Total root length was 35% greater and visible root length was 23% greater for plants growing at 25/15ºC than at 35/25ºC. Total root length present in the soil increased with increasing [CO_2] in both low and high temperatures. The increase was 13% greater for the plants growing at 600 µmol mol^{-1} CO_2, and 20% greater for those at 800 µmol mol^{-1} CO_2, as compared with plants growing at 400 µmol mol^{-1} CO_2 although the difference was not significant.

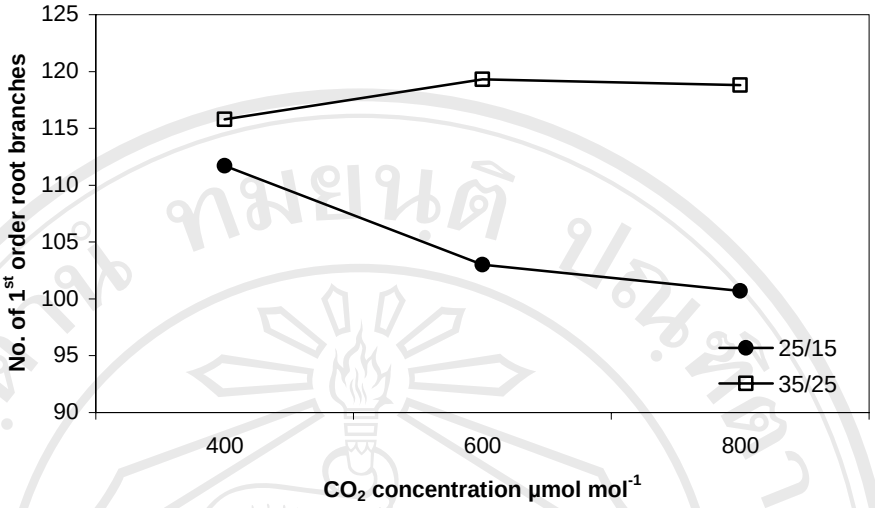


Figure 2.8 Effects of temperature and CO_2 concentration on number of first order branch roots generated from main root axis. LSD (0.05) temperature = 8.89.

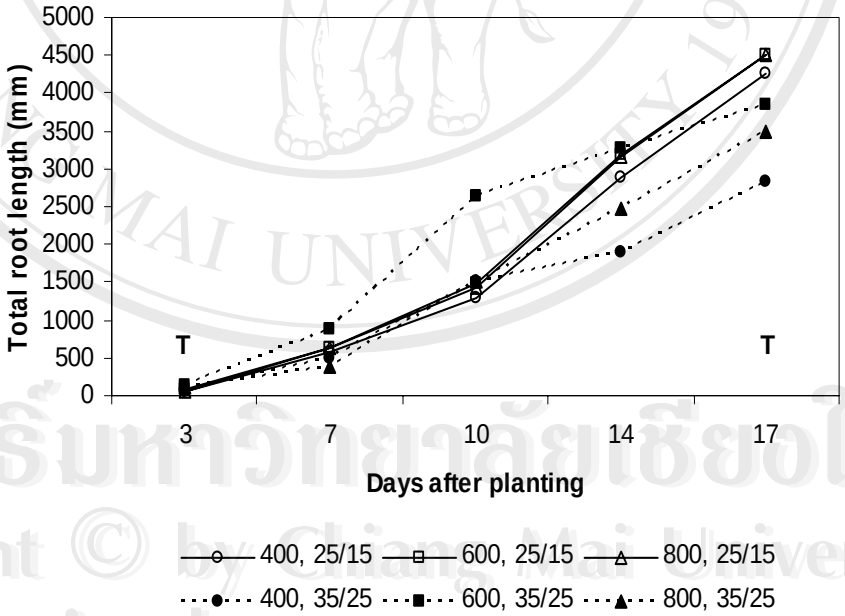


Figure 2.9 Visible root length for different temperature regimes and CO_2 concentrations as observed by minirhizotron camera at different days after planting. Letters indicate significant effects of temperature (T), CO_2 (C), and interaction between temperature and CO2 (T × C), ($P \leq 0.05$).

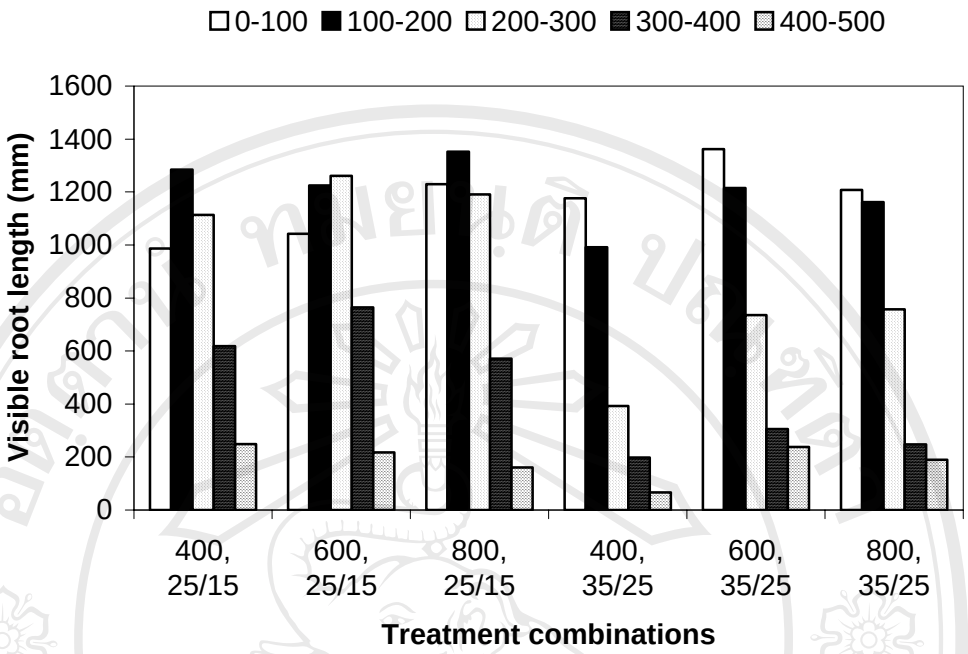


Figure 2.10 Effect of CO_2 concentration and temperature on visible root length as measured by RMS in five 100-mm soil layers.

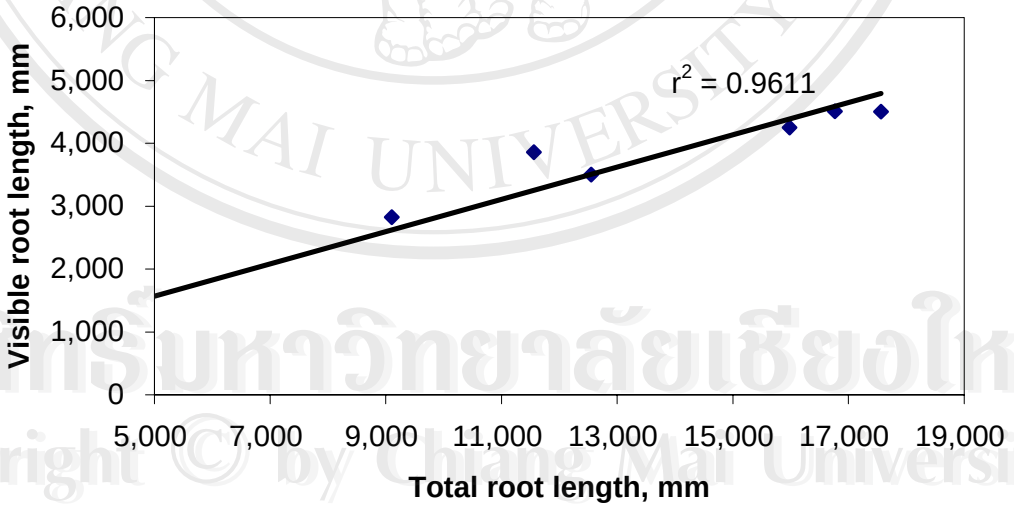


Figure 2.11 Relationship between total root length washed from entire soil and visible root length at the transparent surface of rhizotrons at 17 DAP response to different temperature and CO_2 regimes.

DISCUSSION

Stem elongation

During early growth, plants at 35/25ºC had longer main stems than those at 25/15ºC. Cox (1979) and Ong (1984) demonstrated that the mean optimal temperature range for vegetative growth of peanut is between 25 and 30ºC, while the optimum range for reproductive growth is between 22 and 24ºC. Elevated CO_2 resulted in increasing stem length at 25/15ºC but did length not differ among CO_2 levels at 35/25ºC.

Leaf growth

Individual leaf area and leaf dry weight increased with increasing [CO_2] and specific leaf area decreased as [CO_2] increased in both temperature treatments. Plants grown at 25/15ºC had larger area and dry weight per leaf but smaller in SLA than plants growing at 35/25ºC. These results indicate that plants grown at higher temperatures have thinner leaves which lead to higher in SLA (Wolfe and Kelly, 1992). In contrast, elevated CO_2 has been reported to decrease SLA, which leads to extra palisade layer development (Mousseau and Enoch, 1989), increase mesophyll cell size (Conroy *et al.*, 1986), and increase internal surface area for CO_2 absorption (Radoglou and Jarvis, 1990).

Shoots and pods dry weights

There were significant effects of temperature and CO_2 and their interaction on above ground biomass. Above ground biomass increased with increasing CO_2 level and it was highest in plants grown at 800 μmol mol^{-1} CO_2 in both temperature

treatments. In peanut, it has been recently reported that a doubling of ambient [CO_2] to 700 μmol mol^{-1} enhances leaf photosynthesis by 27% and seed yield by 30% across a range of day-time growth temperature from 32 to 44ºC (Prasad *et al.*, 2003). Chen and Sung (1990) reported that field-grown peanut plants produced more biomass and yielded more pods at 1000 μmol mol^{-1} CO_2 than at ambient CO_2. Stanciel *et al.* (2000) also found that foliage and stem fresh and dry weights increased as [CO_2] increased from 400 to 800 μmol mol^{-1}, but declined at 1200 μmol mol^{-1}. The increased above ground biomass obtained in response to elevated CO_2 in this study agrees with the findings of Chen and Sung (1990) and Sanciel *et al.* (2000).

There was a significant effect of temperature on pod dry weight at different atmospheric [CO_2]. Pod dry weight increased under elevated CO_2 conditions when plants grown under 25/15ºC. Pod dry weight increased 18% and 32% as CO_2 rose from 400 to 600 and 800 μmol mol^{-1}. These results can be explained because elevated atmospheric CO_2 enhances growth and productivity in C_3 plants through its beneficial effects on carbon assimilation and water use (Mulholland *et al.*, 1998). Stanciel *et al.* (2000) reported that the number and the fresh and dry weights of pods increased with increasing CO_2 from 400 to 1200 μmol mol^{-1}. Total seed yield increased in an average of 37%, mature seed by 25%, and dry mass of mature seed by 38%. Prasad *et al.* (2003) also found that elevated CO_2 increased pod and seed yield by about 30% owing to an increase in total number of pods or seeds due to increased photosynthesis and growth. In contrast, elevated temperature adversely affected pod dry weight. In this study, I found that as temperature increased from 25/15 to 35/25ºC, pod dry weight was reduced by 50%. Because the mean optimal air temperature range for vegetative growth of peanut is between 25 and 30ºC, which is warmer than the

optimum range for reproductive growth, which is between 22 and 24ºC (Cox, 1979; Ong, 1984). Both short- and long-term exposure to air and soil temperatures above optimum can cause significant yield loss in peanut (Golombek and Johansen, 1997; Prasad *et al.*, 2000a). Prasad *et al*. (1999, 2000a) observed that day temperature >34ºC decreased fruit-set and resulted in fewer numbers of pods. Decreased fruit-set at high temperatures was mainly due to poor pollen viability, reduced pollen production and poor pollen tube growth, all of which lead to poor fertilization of flowers. Increasing daytime temperature from 23-30 to 34-36ºC reduced the number of subterranean pegs and pods, seed size, and seed yield by 30 to 50% (Cox, 1979; Ong, 1984). Prasad *et al.* (2000b) investigated the effects of daytime soil and air temperature of 28 and 38ºC, from start of flowering to maturity, and reported 50% reduction in pod yield at high temperature.

Root growth

Short-term experiment

In general, a large proportion of root length and numbers were found 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 were found at high temperature. Total root length and number of root were significantly greater in plants grown at low than those at high temperature. Total root length present in the entire soil increased with increasing [CO_2]. This finding agrees with Berntson and Woodward (1992), who concluded that CO_2 enrichment resulted in a more dichotomously branched and longer root system of *Senecio vulgaris* that foraged through larger volumes of soil. Del Castillo *et al.*(1989) reported that elevated CO_2 increased the number of soybean roots but not their elongation rate, from which they inferred that the soil volume explored by the root

system would not increase, but a given volume of soil would be explored more thoroughly.

Long-term experiment

For the long-term experiment, minirhizotron observation showed that total root length, number of roots, and RLD were significantly greater for plants grown under 35/25ºC than 25/15ºC of all CO_2 levels. Increasing atmospheric [CO_2] often results in dramatic increase in root growth. Root dry weight was found to increase under elevated atmospheric CO_2 (Rogers *et al.*, 1994). In this study, I found that fibrous root dry weight increased as CO_2 increased when plants were grown at 25/15ºC treatments, but decreased with increasing CO_2 at 35/25ºC. Belowground responses have been observed in cotton under free-air CO_2 enrichment (Rogers *et al.*, 1992b). Dry weights, lengths and volume of taproots, lateral roots, and fine roots were often higher for CO_2 enriched cotton plants. Rogers *et al.* (1992a) demonstrated enhanced root growth in soybean. Root dry weight, length, diameter, and volume increased when CO_2 was increased; however total root numbers exhibited no response. At high temperature, however, root dry weight was greater than low temperature. This finding could be resulted from there were more total root length, RLD, and root number which observed by minirhizotron camera.

There are conflicting in the results between short-and long-term responses of root. Thus, the greater in total root length at low temperature upon short-term experiment in rhizotrons may not be necessarily indicat 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 disturbance to the soil or roots.

Infection and colonization of peanut by *A. flavus*

The infection of aerial peg by *A. flavus* and the soil *A. flavus* population as evaluated by soil serial dilution technique were found to be greater when plant exposed to 25/15ºC than 35/25ºC temperature. However, the density of this fungus around the root and pod zones as observed by minirhizotron camera and analyzed by QuaCos was greater in 35/25ºC than 25/15ºC.

In this study, peanut plant growing under 25/15ºC temperature had shorter stem length than at 35/25ºC, which may provide the greater opportunity for *A. flavus* conidia movement from the soil surface to infect peanut flowers or aerial pegs. Moreover, plant growing at 25/15ºC also produced much more leaf area than those at 35/25ºC. Thus, when the leaves fall off in to the soil, they provide substrate material for *A. flavus* growth and may lead to enhance aerial peg infection.

In addition, the percent of infection and *A. flavus* population in the soil tended to increase with increasing CO_2 level. Increasing CO_2 levels had result in greater shoot and root growth of peanut plant in this study. Enhanced plant growth further suggests greater delivery of carbon to soil. The amount of root exudates may also increase if root growth is enhanced when plants are grown under elevated atmospheric CO_2, and thus greater substrate for *A. flavus* consumption.

In summary, the results from this study suggest that under two differing 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, while the number of branch was similar among plants grown under those treatment combinations. At low temperature (25/15ºC), leaf area was increased as CO_2 increased whereas specific leaf area was declined. High temperature

(35/25ºC) enhanced shoots and roots growth but reduced final reproductive biomass, which could be resulted from increased number of flower abortion and decreased seed size.

This study has clearly shown 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, higher temperatures resulted in significant yield losses. The beneficial effects of increased CO_2 levels on photosynthesis and growth were overwhelmed by the negative effects of high temperature on reproductive growth. Thus, if the 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.