EXPERIMENT 2

Effects of drought on peanut growth and *Aspergillus flavus* infection

INTRODUCTION

Trends in global elevated of atmospheric CO_2 concentration may likely bring higher temperatures, and increased water deficits especially in tropical and subtropical areas (Chaves and Pereira, 1992). Peanut is grown on 19.3 million ha of land area in about 82 countries. More than half of production area is arid and semi-arid regions. Production of peanut is often limited by insufficient water sometime during the growing season. In Thailand, peanut is grown in both the dry and wet season begins in May and ends in October. Dry season is the critical time for Thai farmers since about 80% of total cultivated land depends mainly on rainfall (Lapang *et al.*, 1980). An understanding of mechanisms that could alleviate plant stress from water deficit is important to improve drought tolerance of peanut. One mechanism of drought tolerance is drought avoidance. Drought avoidance occurs when plants are able to maintain their water status at near normal levels with limited water supply (Subbarao *et al.*, 1986). Drought avoidance can be achieved by maximizing water uptake, minimize losses and maintain water status through an improved rooting depth or density, leaf rolling, phototropic responses, recessed stomata and reflective leaf surfaces (Turner, 1986). Drought resistance in crop is associated with deep root systems, larger root density, and greater extraction of water from deeper soil profile (Senthong and Pandey, 1998; Boonpradub, 2000). Pateña (2000) observed that under water deficit, peanut genotypes that take up water from deeper soil layers can continue growth processes longer than those with shallow root systems. Root growth responses during periodic drought conditions may explain differences in drought tolerance among peanut genotypes. Measurements of root length at regular intervals

throughout the season and at different soil depths and soil moisture regimes may provide a more accurate basis for the analysis of plant growth responses to water deficit.

In addition, drought during reproductive growth also leads to increased *A. flavus* infection and subsequent aflatoxin contamination in peanut (Sander *et al*., 1993; Holbrook *et al.*, 1994). Drought, especially during the later part of the growing season, and temperatures from 25 to 38°C has been associated and greater levels of *A. flavus* infection in peanut. Several studies have been conducted to understand the predisposition of peanut to aflatoxin contamination after drought (Sanders *et al*., 1993; Holbrook *et al.*, 1994) and on screening peanut germplasm and identification of sources of resistance to invasion by *A. flavus*.

If drought increases peanut plant susceptibility to *A. flavus* infection and aflatoxin contamination, then the mechanism for aflatoxin resistance could potentially involve some biochemical or physiological function of the plant. Preharvest aflatoxin resistance of the peanut cultivar Southern Runner has been attributed to its relatively good drought tolerance (Cole *et al.*, 1993). This resulted from reduced metabolic activity due to a decline in pod water content, which increases susceptibility of peanut to fungal invasion. Wooton and Strange (1987) found that resistance of peanut seed to *A. flavus* invasion was correlated with the capacity to synthesize phytoalexins. Dorner *et al*. (1989) demonstrated that seed water activity appeared to be the most important factor controlling the capacity of seed to produce phytoalexin. Moisture deficit and high temperature appeared to cause moisture loss from seed associated with preharvest aflatoxin contamination.

Tannins and calcium (Ca) are components of peanut hulls and seed coats that have been associated with resistance to diseases (Azaizeh and Pettit, 1987; Pitt *et al.*, 1991). Seed coat is a physical barrier to fungal infection of seeds. Pitt *et al.* (1991) found that a higher Ca content in seed coats decreased the growth of *A. flavus* during field-drying. Fernandez *et al.* (1997) also found that the development of *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. were decreased and even suppressed when the Ca content of seed coat increased from 2.2 to 5.5 g kg^{-1}. Amaya *et al.* (1989) reported a possible relationship between resistance to *A. flavus* and total soluble amino compounds and arabinose content among peanut cultivars. Differences among cultivars were found in the elemental composition of the seed coat. The seed coat of resistant peanut cultivar, PI 337409, contained intermediate levels of phosphorus and sulfur, and higher level of calcium. Extractable antibiotic phenols, especially tannins, have been reported to be present constitutively in peanut seed coats and are thought to inhibit *A. flavus* (Daigle *et al.*, 1984). According to Polles *et al.* (1981), tannin concentration in pecans ranged from 0.70 to 1.71%. These compounds inhibit the growth of several microorganisms and confer a protective mechanism to the plant. Azaizeh and Pettit (1987) reported that level of tannin compounds in peanut seed coats and cotyledons differed among peanut genotypes, with tannin concentrations generally higher in seed coats than in cotyledons. Carter (1970) reported that peanut tannin extracts from colored seed coats inhibited the germination of *A. flavus* spores. Sanders and Mixon (1978) isolated a crude tannin extract from peanut seed coat that exhibited fungistatic activity toward actively growing *A. parasiticus* and could be related to colonization by this fungus.

Thus, the changes of mineral elements and tannin content in peanut hulls and seed coats as affected by drought may relate to resistance or susceptibility of peanut genotypes to *A. flavus* infection. However, studies on the effect of drought on condensed tannin, mineral elements, crude fats, and crude protein contents associated with *A. flavus* infection are limited.

These studies aim: i) to evaluate effects of different levels of water deficit on shoots and roots growth of peanut genotypes, ii) to determine the responses of peanut genotypes to *A. flavus* infection under different levels of water deficit, and iii) to determine the biochemical responses of pods of peanut genotypes to *A. flavus* infection under drought conditions.

MATERIALS AND METHODS

Green house research was conducted at the Georgia Envirotron, at the Griffin Campus of the University of Georgia, USA during 2002 and at Maehea Agricultural Research Station and Training Center, Chiang Mai, Thailand during 2004.

Sub-experiment 1: Effect of drought duration on peanut growth and *Aspergillus flavus* infection

Forty-eight 214-L containers were arranged in a four by three factorial completely randomized design with four water treatments with three peanut genotypes and with four replications in a green house of the Georgia Envirotron, the University of Georgia. Minirhizotron tubes were installed horizontally along the diameter of each container with top of the tubes at 5, 25, 50, and 75 cm depths from the soil surface. Shielded thermocouples and soil moisture blocks (Model 223 Delmhorts, Cambell Scientific, Logan, Utah, U.S.A.) were installed at 5, 25, and 75 cm depths (Appendix D). A Campbell Scientific CR10X datalogger was used to log data every hour from thermocouples and soil moisture blocks throughout the experiment.

The three peanut genotypes used in the experiment were 511CC, drought tolerant and resistant to pre-harvest aflatoxin contamination (PAC); 419CC, drought and PAC susceptible; and Tainan 9, unknown drought and PAC resistance, which is a commercial variety from Thailand. Seeds of similar size and weight were selected within each genotype. Seeds were pre-germinated in moist germination paper at 30^{o}C for five days. Four healthy germinated seeds of each genotype were planted in containers filled with Tifton loamy sand soil (86% sand, 8% clay, and 6% silt).

Containers were placed in green house, set to 35/25°C day/night. All containers were irrigated lightly by hand at 1- to 2-day intervals until seedlings established. After establishment, containers were watered twice weekly with half-strength Hoagland's solution until drainage occurred from the bottom of each container.

The four irrigation treatments were: water application at 3- to 4-day intervals as a control treatment (T1), 7-day intervals (T2), 14-day intervals (T3), and 21-day intervals (T4). All treatments started with water saturated soil with half-strength Hoagland's solution using an automatic irrigation system at 35 DAP. Thus, total numbers of irrigation cycles were 24 for T1, 12 cycles for T2, 6 cycles for T3, and 4 cycles for T4 plants until final sampling. Half-strength Hoagland's solution was applied instead of tap water at 35, 56, 77, and 98 DAP for T1, T2, and T4, and at 35, 49, 77, and 105 DAP for T3. Insecticides were sprayed to prevent incidence of white fly (*Bemisia tabaci* Gennadius) and red spider mites (*Tetranychus urticae* Koch). Plants grew from 21 May to 18 September 2002 for a total growth period of 120 days.

Inoculum preparation

Two strains of *Aspergillus flavus*, which are known to produce a green fluorescent protein (GFP) with yellow green-fluorescence when exposed to UV light, were used. 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. The *A. flavus* cultures were incubated at 30°C for 7 days. Then conidia were washed from the Petri dishes using sterile distilled water. Spore suspension inoculum was refrigerated at 4°C until used.

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 flasks was adjusted to 25% by adding 50 ml spore suspension of *A. flavus*. Flasks were sealed and incubated at 30°C for 7 days. Flasks were shaken daily to provide for uniform distribution and growth of the fungus. Cracked corn inoculum was stored in a refrigerator at 4°C until used.

At 35 DAP, 50 g of cracked corn inoculum was incorporated into the soil surface and mixed with upper 1 cm soil over the uppermost minirhizotron tubes for each container.

Crop growth observation

Shoot traits were observed at 14-day intervals, beginning at 25 DAP. Shoot traits observed were main stem length, number of branches on main stem, width and length of the youngest fully-expanded leaf, number of leaflets, leaf color, and visual stress rating.

Length and width of main stem leaflets were measured and individual leaf area was estimated nondestructively based on a formula for peanut leaves developed by Padalia and Patel (1980).

$$\text{Leaf area} = 0.70 \times \text{length} \times \text{width}$$

Total plant leaf area was obtained by counting all leaflets in the plant and multiplying by an average leaflet size measured from the main stem.

Leaf color was evaluated according to a 1 to 9 visual scoring system in which a score of 1 refers to leaves with very dark green color, 5 refers to yellowing leaves, and 9 refers to brown leaves.

Visual stress levels were rated at 76, 83, 90, 97, and 104 DAP, based on a scale of 1 to 5 (Rucker et al., 1995). Plants with a rating of 1 had leaves raised, leaf angle above horizontal, a bright green color, and no visible stress. Plants with a rating of 5 had folded leaves, drooping stems, and a gray cast.

A minirhizotron camera (BTC100-X, Bartz Technology Corp., Santa Barbara, CA 93101, U.S.A.) was used to observed roots growth. Digital root images were collected at 14-day intervals from minirhizotron tubes installed at six depths. Images were analyzed using RMS software (Ingram and Leers, 2001). Data on root length, diameter, and number were obtained for each image.

At 115 DAP, a minirhizotron camera with built in UV lighting system 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 (Pateña and Ingram, 2000). All images were analyzed for green fluorescence color of *A. flavus* fungus by Quantitative Analysis of Color System (QuaCos) program.

Shoots and pods were harvested by hand at 120 DAP. Leaves of plants in each container were separated and the area of a 100-leaflets subsample was measured using a leaf area meter (Model Li-3000, Li-Cor, Inc., Lincoln, NE). One plant from each container was separated into stems and leaves, and then weighed fresh. The three remaining plants were combined for whole shoot fresh weight measurement. All samples 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. Twelve fresh pods from each container were sampled, then cleaned thoroughly in tap water to remove access soil and kept in plastic bags for determine presence of *A. flavus*. The

others were dried at 70°C for 72 hr and then recorded dry weight. Each of the 12 subsample pods was surface sterilized for 60 s in 30% Clorox solution, rinsed twice in sterilized water, and then placed on sterilized paper towels to remove excess water. Pods were separated into pod shells and seeds, then 12 seeds and 12 half pod shells were plated on M3S1B medium. Cultures were incubated at 30°C for 5 days. Results were recorded as percent of infection for seed and pod shell.

Soil was collected from each container at 120 DAP. Soil samples were air dried for 24 h and sieved trough 2 mm mesh screen. Then, 10 g of each sample was suspended in 90 ml sterile water in a 200-ml bottle. The suspension was thoroughly mixed by shaking for 15 min. A sterile pipette was used to transfer 1 ml of suspension to a tube containing 9 ml of sterile water. Then, a fresh sterile pipette was used to mix and transferred 1 ml of this suspension to a second tube containing 9 ml of sterile water. This process continued until the number of dilutions reached to 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. Plates were incubated at 30°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.

Sub-experiment 2: Biochemical responses of peanut pods to drought and *Aspergillus flavus* infection

This experiment was conducted at the farm of Maehea Agricultural Research Station and Training Center, Chiang Mai University, Chiang Mai, Thailand, from 14 October 2004 through 11 February 2005, a total of 120 days. The average daily maximum temperature was 31.0 °C and the average daily minimum temperature was 17.1 °C, with and average relative humidity of 72.0%. A total precipitation of 67.7 mm was recorded during the experiment. The soil was a sandy loam (75% sand, 7% silt, and 18% clay) with pH = 6.35, containing 25.8 g kg^{-1} organic matter, 1.30 g kg^{-1} N, 0.048 g kg^{-1} P, 0.24 g kg^{-1} K, 1.18 g kg^{-1} Ca, and 0.006 g kg^{-1} Zn. Three peanut genotypes, 511CC (drought and aflatoxin resistant), 419CC (drought and aflatoxin susceptible), and Tainan 9 (commercial variety in Thailand), were used. Seeds were pre-germinated in moist germination paper at 30°C for five days. Nine uniform, healthy germinated seeds of each genotype were planted in 200-L cylindrical containers filled with sandy loam soil with 1 seed $hill^{-1}$ at a spacing of 20 × 25 cm (Figure 3.1). Treatment combinations (three peanut genotypes, two water regimes and two inoculation methods) were arranged in a completely randomized design with three replications. At 10 DAP we applied 156 kg ha^{-1} of 12-24-12 of NPK fertilizer. Carbosulfan insecticide [2-3-dihydro-2, 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate] was used for insect control whenever necessary.

Figure 3.1 Nine peanut plants growing in a 200-L cylindrical container (Dimension: 80 cm diameter × 40 cm high), filled with sandy loam soil with 1 plant $hill^{-1}$ at a spacing of 20 × 25 cm.

Inoculum preparation and application

Spore suspension: Two strains of *Aspergillus flavus* modified to produce a green fluorescent protein (GFP) that fluoresces when exposed to UV light were used. One strain was produced by Gary Payne (North Carolina State University, Raleigh, NC) and other by Jeffrey Carrey (USDA-ARS, New Orleans, LA). While these two *A. flavus* strains may differ in relative pathogenicity and location of the GFP gene (J. Carrey, 1999, personal communication; G. Payne, 2000, personal communication), this study did not intend to investigate the difference between the strains. Instead, I applied both strains to increase the overall probability of infection. The two *A. flavus* strains were maintained separately 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 L 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 measured with a hemacytometer and adjusted with sterile distilled water to 4×10^6 conidia ml^{-1}. Inoculum was refrigerated at 4°C until use.

At 36 DAP, 18 containers (6 containers/genotype) were inoculated with 120 g of cracked corn inoculum (previously described in sub-experiment 1), followed by spraying spore suspension of GFP *A. flavus* through the shoots and flowers (F+), while the other 18 containers received no fungus (F0). In well-watered treatment (WW), plants were watered by hand until drainage appeared from the bottom of each

container every other day until harvest. Plants in water-deficit treatment (WD) received normal irrigation except for the period from 50 to 100 DAP when irrigation was withheld to impose water deficit. From 100 DAP until harvest, plant received regular irrigation. Peanut plants were harvested at 120 DAP, pods were removed from each plant by hand immediately after shoot harvesting. Fifteen fresh pods from each container were sampled, then cleaned thoroughly in tap water to remove excess soil and kept in plastic bags for determination of the presence of GFP *A. flavus*. Each fifteen pods were surface-sterilized for 60 s in 30% Clorox solution, rinsed twice in sterilized water and then placed on sterilized paper towels to remove excess water. Pods were separated into pod shells and seeds, then 15 seeds and 15 half-pod shells were placed on Petri dishes containing M3S1B medium. Cultures were incubated at 30°C for 5 days. Results were recorded as the percent of infection for seed and pod shell. Pod shell and seed coat were also analyzed for content of condensed tannin (CT) and minerals associated with disease resistance, namely, Ca, Mg, Fe, Zn and Mn. Cotyledons were analyzed for contents of crude protein and fat.

Sample preparation for minerals, tannins, crude protein and fat analysis

Peanut pods of each treatment were cleaned and then oven-dried at 60^{o}C for 48 h. After drying, pods were separated into pod shell, seed, and seed coat. These tissues were then ground using a micro hammer mill (PX-MFC, Kinetica, Switzerland) and sieved through 1 mm mesh screen. Samples were refrigerated at 4^{o}C until subsequent analysis.

Mineral analysis

Finely-ground plant tissue was weighed into 1.00 ± 0.05 g samples and placed into porcelain crucibles. Samples were ashed overnight in a muffle furnace at 450°C. After cooling, ashed samples were dissolved in 5 ml of 20% (2N) analytical grade hydrochloric acid (HCl). Solution was warmed if necessary tp completely dissolve the residue. The solution was filtered through acid-washed filter paper into a 50-ml volumetric flask. The filter paper was rinsed with hot water into the volumetric flask and the total volume brought to 50 ml using distilled, deionized water. For calcium analysis, 0.2% lanthanum chloride was added to both sample and standard solutions to overcome interference from silicon, aluminum, phosphate, and sulphate. Contents of Ca, Mg, Zn, Fe, and Mn were measured with a flame atomic absorption spectrophotometer (AAS Model; Perkin-Elmer 3100, USA) with data converted to g kg^{-1}.

Tannin analysis

Condensed tannins (CT) in pod shells and seed coats were extracted from finely-ground samples (200 mg) of each peanut genotype with 10 ml of aqueous acetone (70:30; v/v) in Erlenmeyer flasks for 24 h at room temperature with occasional mixing by swirling as described by Makkar *et al.* (1996). After extraction, the supernatant of each sample was decanted into a conical flask and mixed thoroughly. A 1-ml (pod shell) or 30-µl (seed coat) aliquot of the supernatant from each sample was added to 6 ml of 80% (v/v) butanol-HCl reagent in duplicate test tubes. Tubes were capped and transferred to a dry block heater that had been pre-heated and maintained at 100°C. Tubes were heated for 1 hr, then removed and cooled before decanting samples into vials. Absorbance was read at 550 nm, using a

Cecil CE 2021 single- beam spectrophotometer (Model 2000, Cecil Instruments, UK) to estimate condensed tannin concentration. Blank samples containing the reagent were included in the measurements. Content of CT was expressed as A_{550} g^{-1} sample.

Crude protein analysis

Crude protein content in cotyledon of peanut was determined by the Kjeldahl method (AOAC, 1990). Finely-ground 0.5-g samples were placed into Kjeldahl digestion flasks. Then, 16.7 g K_2SO_4, 0.01 g anhydrous $CuSO_4$, 0.6 g TiO_2, 0.3 g pumice, 0.5 g Alundum granules, and 20 ml H_2SO_4 were added into each flask. For digestion, flasks with mixture were brought to a rolling boil at 370-400°C until dense white fumes cleared the bulbs of the flasks. Flasks were swirled gently, with continued heating for an additional 40 min. After cooling we added 250 ml distilled water. Titration beakers were prepared by adding 25 ml of 0.05 M H_2SO_4 standard solution, 100 ml distilled water, and 3 drops of methyl red indicator solution (dissolved 1 g methyl red in 100 ml methanol). Then, slowly down side of digestion flask we added 100 ml NaOH solution and immediately connected flask to distillation apparatus, mixed completely, and distilled at 7.5-min boil rate until ≥ 150 ml distillate was collected in a titration beaker. We titrated excess standard acid in distillate with 0.1 M NaOH standard solution and corrected results using analyses from blank samples. We calculated N, in %, as follow:

$$\mathrm{N} = \frac{\left[\left(M_{acid}\right)(2)\left(ml_{acid}\right) - \left(ml_{blank}\right)\left(M_{NaOH}\right) - \left(ml_{NaOH}\right)\left(M_{NaOH}\right)\right]}{sample\ weight\ (mg)} \times 1400.67$$

Where; ml_{NaOH} = Volume of standard base used for titration

ml_{acid} = Volume of standard acid used as trapping solution

ml_{blank} = Volume of standard base needed to titrate 1 ml standard acid minus volume of standard base needed to titrate reagent blank carried through method and distilled into 1 ml standard acid.

M_{acid} = Molarity of standard acid

M_{NaOH} = Molarity of standard base

Crude protein (CP) = N × 6.25

Crude fat analysis

Crude fat in cotyledons of peanut was determined by Soxhlet extraction method (AOAC, 1990), using anhydrous ether as the non-polar solvent. For each sample 2 g was placed on filter paper in a funnel and rinsed with five 20-ml portions of water to remove soluble components that may interfere with fat extraction. Then, samples were dried at 100°C for 5 hr and cooled in a desiccator. After drying, samples were transferred to extraction thimbles and placed in Soxhlet extraction apparatus. We added 2-3 pumice stone to the round bottom of the receiving flask, dried, weighed, and connected to the extraction flask. The upper end of the extraction flask was connected to a water condenser and we poured 150 ml ether through the opening at the top of the condenser. Flasks were heated on hot plates adjusted give a condensation rate of 2 to 3 drops s^{-1} and extracted for 16 hr. When the extraction was complete, the apparatus was disconnected to remove the extraction and receiving flasks. Then, we placed the receiving flask in an oven for 30 min at 100°C, cooled in desiccator, and weighed.

Calculation:

$$\text{Crude fat} = \frac{weight\ of\ \ fat}{weight\ of\ \ sample} \times 100$$

Statistical analysis

Statistical analyses were performed using SAS statistical package, Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA.). Means were compared by least significant difference (LSD). Unless otherwise stated, all significant differences were tested at $P \leq 0.05$. Simple correlation coefficients among all parameters were calculated.

RESULTS

Effect of drought duration on peanut growth and *Aspergillus flavus* infection

Soil water potential

Soil moisture potential measurements at three soil depths for three peanut genotypes growing under four different water regimes are shown in Figure 3.2-3.5. There were missing observations from 50 to 64 DAP and from 71 to 78 DAP for plants receiving T1 water treatment as a result of failures in datalogger storage.

In plants receiving T1 water treatment, soil moisture potential was always near field capacity at 25 and 75 soil depths, while at 5 cm depth soil water potential reached a minimum of -0.10 MPa at 82 DAP in all three peanut genotypes (Figure 3.2). Otherwise, soil water potential remained at or near field capacity until the end of growing periods.

In T2 treatment, soil water potential differed among soil depths from 40 DAP until 110 DAP (Figure 3.3). At 75 cm soil depth, soil water potential was near field capacity, except from 61 to 82 DAP in genotype 419CC and 511CC when lower soil water potentials were observed. At 25 cm depth, soil water potential varied between 0 and -0.05 MPa for 511CC and between 0 and -0.07 MPa for 419CC and Tainan 9. At 82 DAP, soil water potential at this soil depth reached -0.09 MPa in 511CC and Tainan 9, whereas it reached -0.011 MPa in 419CC. At 5 cm soil depth, soil water potential was generally stable during the growth period, which varied between -0.11 and -0.13 MPa for 419CC and Tainan 9 and between -0.09 and -0.11 MPa for 511CC. Moreover, soil moisture potential of all genotypes reached to a minimum (-0.12 to -0.14) by 85 DAP.

In T3 treatment, six cycles of water deficit were imposed (Figure 3.4). Soil water potential at 75 cm depth was near field capacity, except from 57 to 64 DAP and from 85 to 92 DAP, when soil water potential reached -0.1 MPa for all peanut genotypes. At 25 cm soil depth, all peanut genotypes maintained a soil water potential between -0.1 and -0.2 MPa. At 5 cm soil depth, the minimum -0.2 MPa was observed for 419CC and -0.3 MPa for Tainan 9 at 62 and 90 DAP. For genotype 511CC, water deficits were greater than for the other genopytes at 62 and 90 DAP with soil water potential reaching -0.7 to -0.8 MPa.

In T4 treatment, four cycles of water deficit were imposed during the experiment (Figure 3.5). Soil water potentials differed levels among soil depths at 56, 77, and 98 DAP, with a soil water potential at 5 cm soil depth of -0.2 MPa. At 25 cm soil depth, soil moisture deficit was greater for 419CC than for Tainan 9 and 511CC, with a soil water potential reached -0.9 MPa for 419CC, while the other two genotypes maintained at -0.4 MPa by 77 DAP. At 5 cm soil depth, water deficit was greatest for genotype 511CC, which had a soil water potential minimum of -1.5 MPa at 77 DAP, as compared with minimum soil moisture potentials of -1.0 MPa for 419CC and Tainan 9.

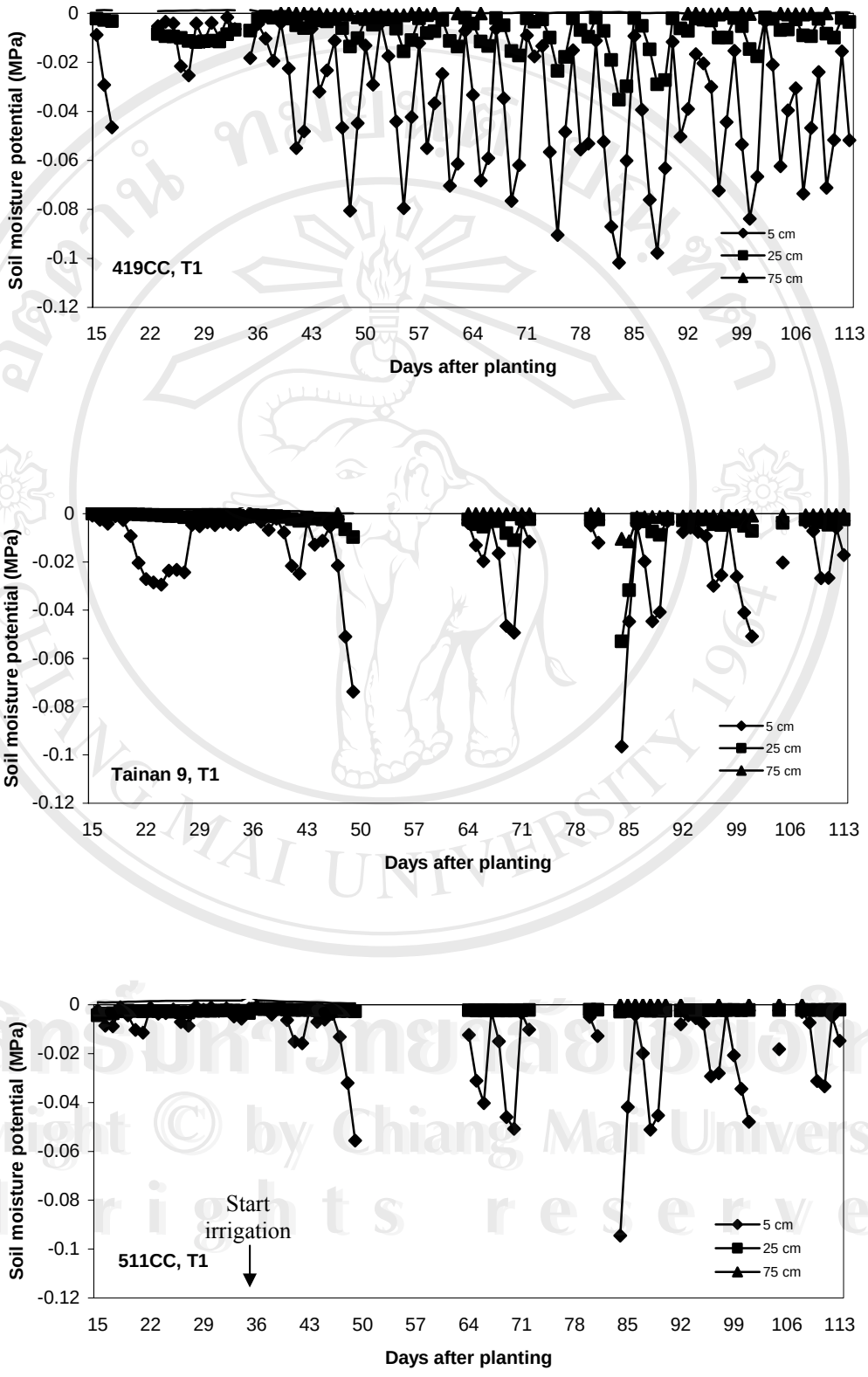

Figure 3.2 Soil moisture potential at three depths through 24 cycles for 3 peanut genotypes. Water applied at each 3-to 4-day intervals (T1).

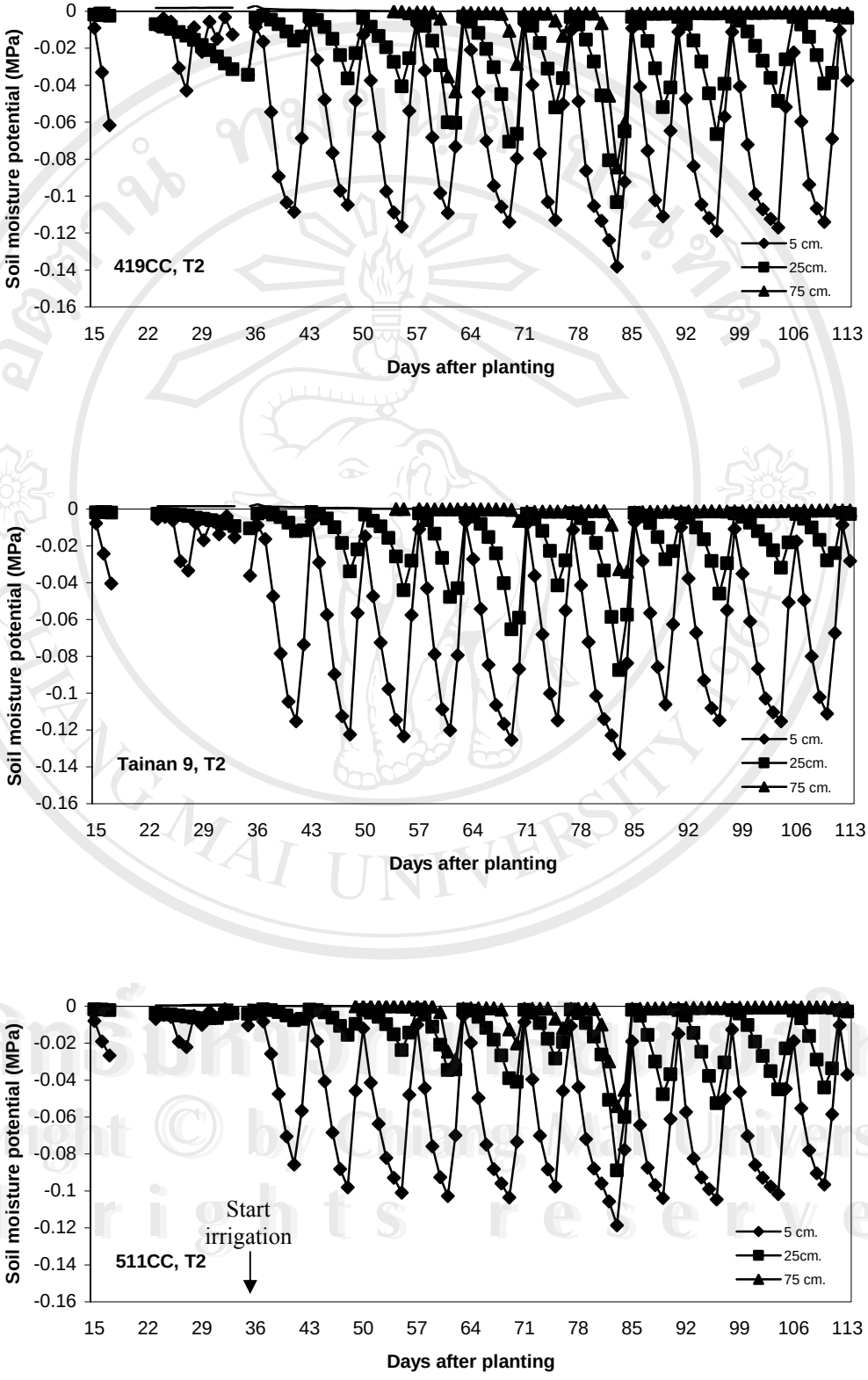

Figure 3.3 Soil moisture potential at three depths through 12 cycles for three peanut genotypes. Water applied at 7-day intervals (T2).

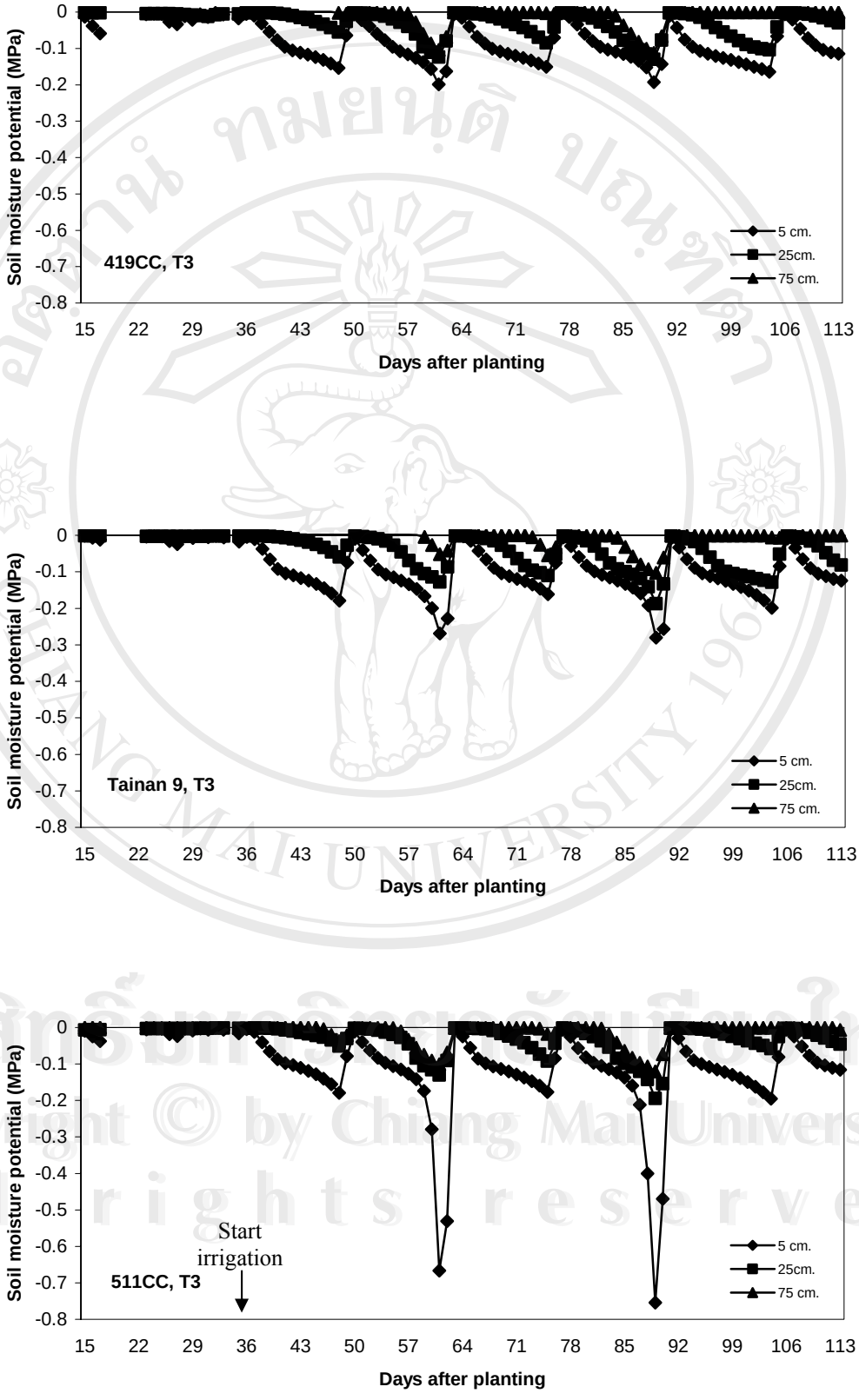

Figure 3.4 Soil moisture potential at three depths through 6 cycles for three peanut genotypes. Water applied at 14-day intervals (T3).

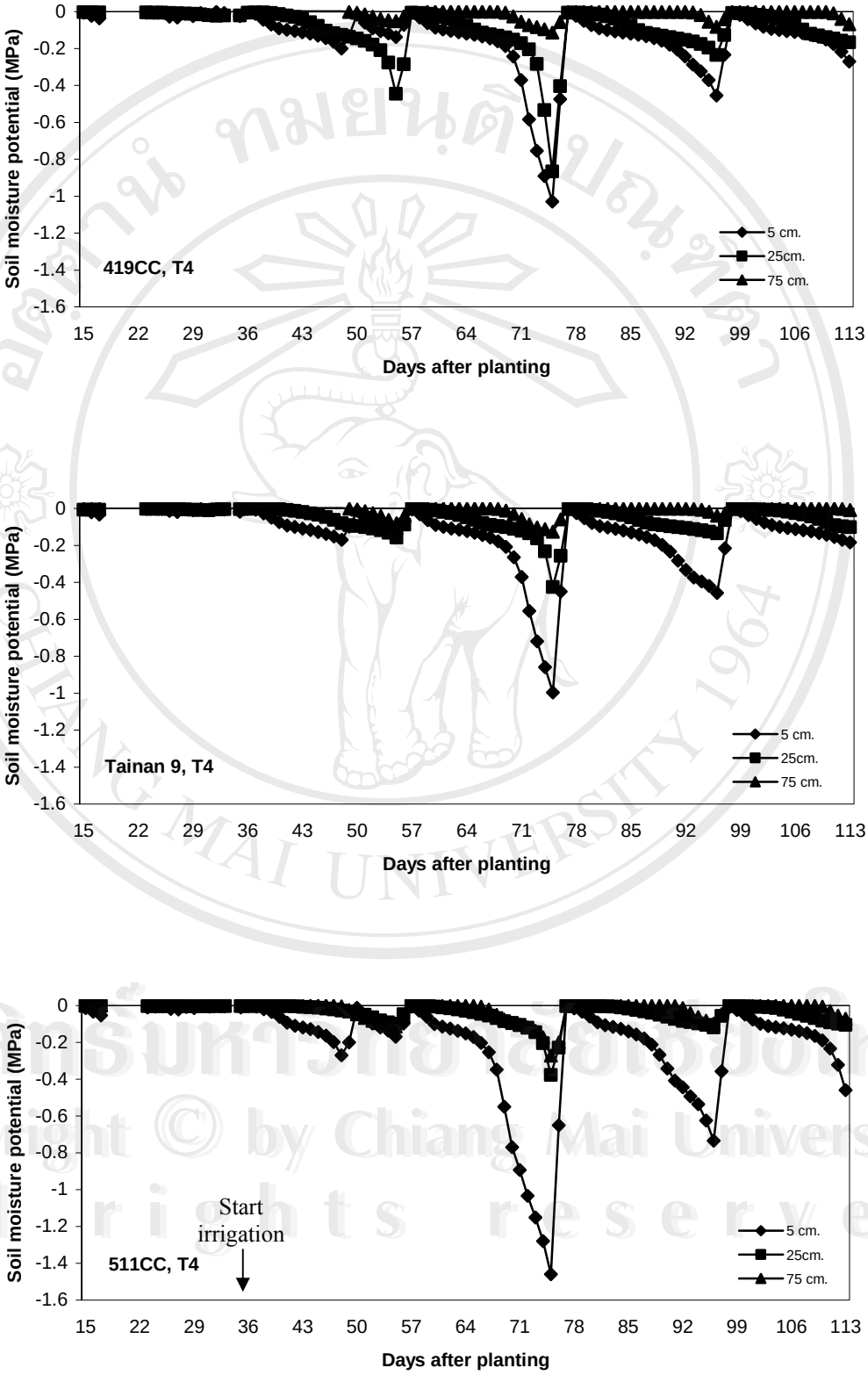

Figure 3.5 Soil moisture potential at three depths through 4 cycles for three peanut genotypes. Water applied at 21-day intervals (T4).

Shoot growth

Leaf growth

Individual leaf area was estimated by non-destructive measurement was not significantly affected by water treatments. Tainan 9 had larger leaflets than 419CC and 511CC (Table 3.1).

There were significant differences in leaflet numbers among genotypes. Genotype 511CC had most leaflet followed by 419CC and Tainan 9. Significant effects of water treatment on total leaflets plant^{-1} were observed from 39 to 109 DAP, and but the interaction between water treatment by genotype was significant only at 81 DAP (Table 3.1).

Significant effects of water treatment on total leaf area were observed at 67, 81, 95, and 109 DAP, whereas genotype by water treatment interactions were significant found only at 81 and 109 DAP (Table 3.1). Total leaf area significantly differed among peanut genotypes at 53, 67, 81, and 109 DAP. Total leaf area plant^{-1} was greatest in 511CC, while 419CC and Tainan 9 had similar mean total leaf area. Although 511CC had the smallest leaflet size, it also had the most leaflets among the three genotypes, which resulted in largest leaf area plant^{-1} overall of observations.

Comparing water treatments, total leaflet number and leaf area plant^{-1} did not differ between T1 and T2, whereas T3 and T4 significantly reduced leaflet numbers from 67 to 109 DAP. T3 and T4 also significant reduction in total leaf area during the same periods.

Tainan 9: Total leaflet number and leaf area differed significantly among four water treatments at 53 to 109 DAP (Figure 3.6 A-B). Total leaf area was greatest in T2 treatment and least in T4 treatment. Watering at 21-day intervals significantly

reduced total leaf area by 35% at 53 DAP, 29% at 67 DAP, 59% at 81 DAP, 70% at 95 DAP, and 72% at 109 DAP. Reductions in leaf area by the T3 treatment were also observed at 81 to 109 DAP. Reductions in leaf area were 27% at 81 DAP, 37% at 95 DAP, and 41% at 109 DAP. Leaf areas were not significantly different for T1 and T2 treatments.

419CC: There was no statistically significant difference in leaflet number among water treatments for any observation time. There were leaf area differences between water treatments at 67 and 95 DAP. Leaf area in T4 treatment was 46% less than in T1 treatment at 81 DAP and 52% less at 95 DAP (Figure 3.7 A-B).

511CC: Water treatments significantly affected leaflet numbers and leaf areas from 39 to 109 DAP, except at 53 DAP. Leaflet number reductions in T4 treatment compared with T2 treatment from 39 to 67 DAP, and compared with T1 treatment from 81 to 109 DAP were 44% at 39 DAP, 34% at 67 DAP, 60% at 81 DAP, 51% at 95 DAP, and 48% at 109 DAP. Comparing between T3 and T1 treatments, leaflet numbers were reduced by 40% at 81 DAP, 58% at 95 DAP, and 62% at 109 DAP (Figure 3.8 A).Total leaf area did not differ significantly between T1 and T2. At 39 DAP, leaf area was 54% larger in T2 than T4 and 39% larger than T3. At 81 DAP, the greater leaf area of T1 plants were 65% than T2 and 42% than T3 plants. At 95 DAP, the reduction in leaf area was similar between T3 and T4 treatments. Leaf area was reduced as much as 49 to 50% compared with T1 treatment. At 109 DAP, the reductions in leaf area were 52% for T4 and 63% for T3 as compared with T1 treatment (Figure 3.8 B).

Water treatment significantly affected leaf color at 76, 83, 90, and 97 DAP. Plant receiving T1 and T2 treatments maintained green leaf color with score less than

five throughout the experiment, whereas those receiving T4 and T3 treatments showed light brown leaves at 76 and 90 DAP, respectively, with leaf color scores from seven to eight. There was no statistically significant difference in leaf color among genotypes. There was, however, an interaction between water treatment and genotype for leaf color at 76, 83, and 97 DAP.

Plants receiving T1 water treatment maintained leaves raised and bright green color and no stress with the score less than 2 during the growing period. No stress symptoms occurred in T2 treatment with the score was between 2 to 3, except at 83 DAP when plants slightly wilted. Severe stress was observed in T3 treatment with a score of 5 at 90 DAP for all genotypes (Appendix E). In T4, treatment showed less symptoms of stress than T3 treatment, with a score between 2 and 4.5.

Stem growth

Main stem growth of three peanut genotypes differed in response to water regimes, and effects became greater as plant age increased from 25 to 109 DAP (Table 3.1). Tainan 9 generally had longer stems than 419CC and 511CC throughout the experiment regardless of water treatment. Effects of water treatment on stem elongation were significant from 53 to 109 DAP. The longest main stem was found in T1 treatment. Plants receiving T3 treatment had significantly shorter stems than T1 treatment by 17% at 81 DAP and 22% at 95 DAP. While stem length reductions in T4 treatment was 28% at 53 DAP, 29% at 67 DAP, 35% at 81 DAP, 37% at 95 DAP, and 37% at 109 DAP.

Tainan 9: From 39 to 109 DAP, main stem lengths of T1 treatment was significantly longer than those of the other water regimes Stem length reductions in

T3 plants were increased from 19 to 34%, whereas it was increased from 23 to 48% in T4 plants as compared with T1 plants (Figure 3.9 A).

511CC: Main stem length of T1 and T2 treatments were significantly longer than T3 and T4 treatments (Figure 3.9 B). Comparing between T2 and T3 treatments, stem lengths were reduced by 22 to 30% from 39 to 100 DAP, whereas stem length reduction ranged from 29 to 35% in T4 treatment.

419CC: There were no statistically significant stem length and branch number difference between water treatments at any age of observations. The stem length of T1, T2, and T3 treatment s were similar and longer than T4 treatment (Figure 3.9 C).

Table 3.1 Significant differences for plant traits measured at 25 to 109 days after planting (DAP).

DAP	Plant traits	Source of variation		
		Genotype	Water treatment	Interaction
25	Individual leaf area, cm^2	**	NS	NS
	Leaflet number $plant^{-1}$	**	NS	NS
	Total leaf area, cm^2	NS	NS	NS
	Main stem length, cm	**	NS	NS
39	Individual leaf area, cm^2	**	NS	
	Leaflet number $plant^{-1}$	**	*	NS
	Total leaf area, cm^2	NS	NS	NS
	Main stem length, cm	**	NS	NS
53	Individual leaf area, cm^2	*	NS	NS
	Leaflet number $plant^{-1}$	**	*	NS
	Total leaf area, cm^2	*	NS	NS
	Main stem length, cm	**	**	NS
67	Individual leaf area, cm^2	**	NS	*
	Leaflet number $plant^{-1}$	**	**	NS
	Total leaf area, cm^2	*	**	NS
	Main stem length, cm	**	**	NS

Table 3.1 Continue

DAP	Plant traits	Source of variation		
		Genotype	Water treatment	Interaction
81	Individual leaf area, cm^2	**	NS	NS
	Leaflet number $plant^{-1}$	**	**	NS
	Total leaf area, cm^2	*	**	*
	Main stem length, cm	**	**	NS
95	Individual leaf area, cm^2	**	NS	NS
	Leaflet number $plant^{-1}$	**	**	NS
	Total leaf area, cm^2	NS	**	NS
	Main stem length, cm	**	**	NS
109	Individual leaf area, cm^2	**	NS	*
	Leaflet number $plant^{-1}$	**	**	NS
	Total leaf area, cm^2	*	**	*
	Main stem length, cm	**	**	NS

*, ** Significant difference at 0.05, 0.01 level of probability, respectively.

NS, No significant difference.

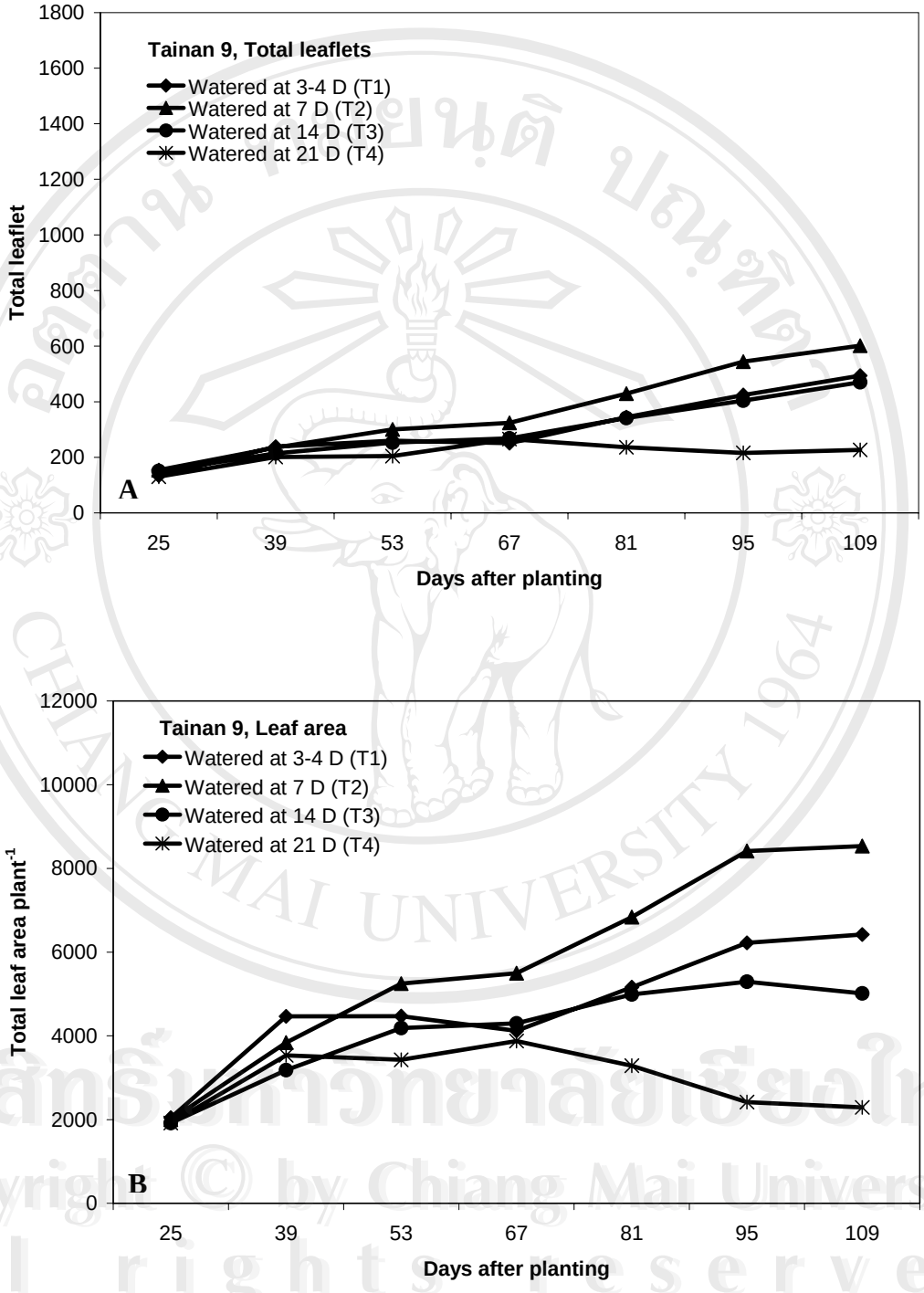

Figure 3.6 Leaflet numbers (A) and total leaf area (B) of Tainan 9 peanut genotype response to four water treatments, as measured by non-destructive method at different plant ages.

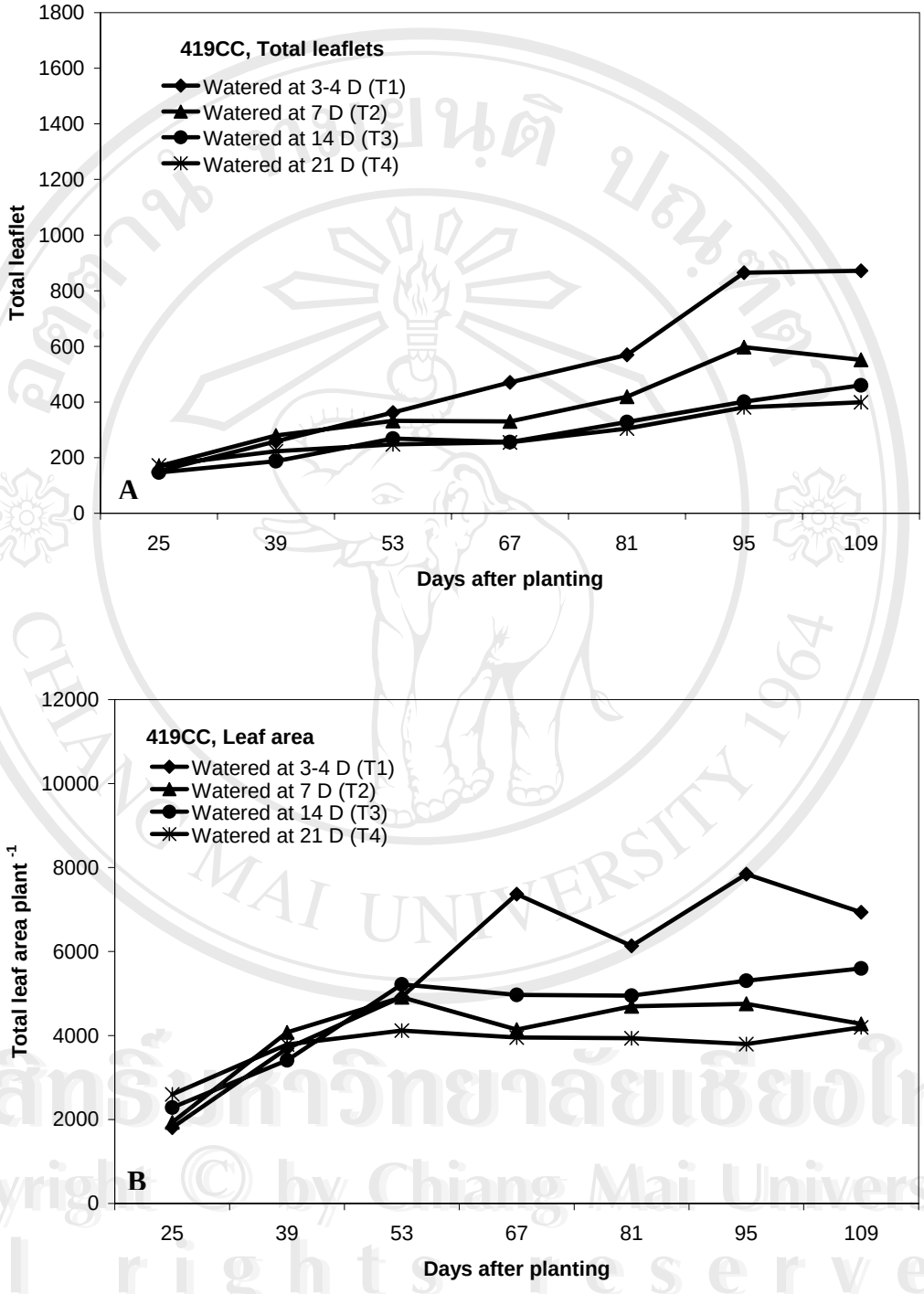

Figure 3.7 Leaflet numbers (A) and total leaf area (B) of 419CC peanut genotype response to four water treatments, as measured by non-destructive method at different plant ages.

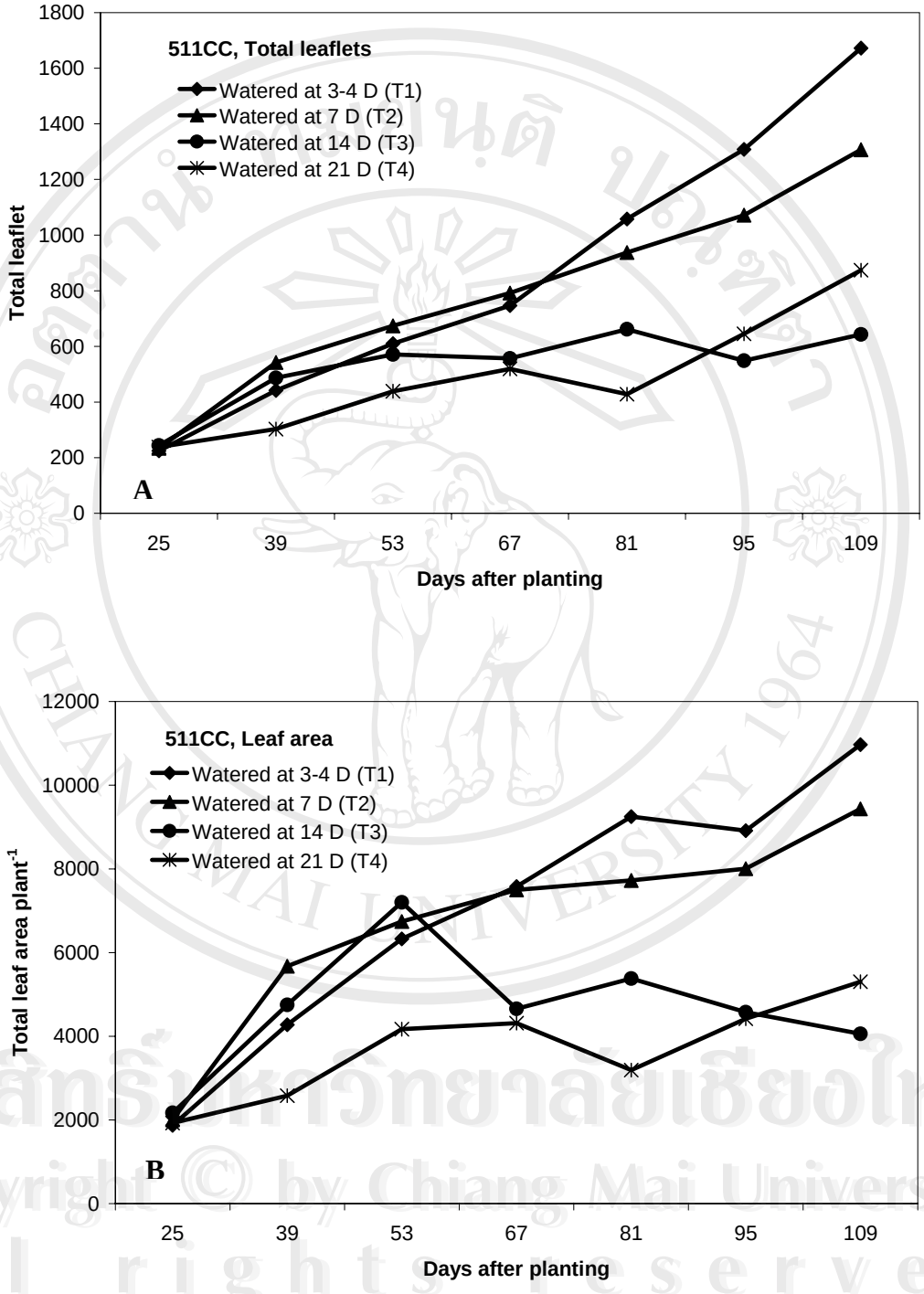

Figure 3.8 Leaflet numbers (A) and total leaf area (B) of 511CC peanut genotype response to four water treatments, as measured by non-destructive method at different plant ages.

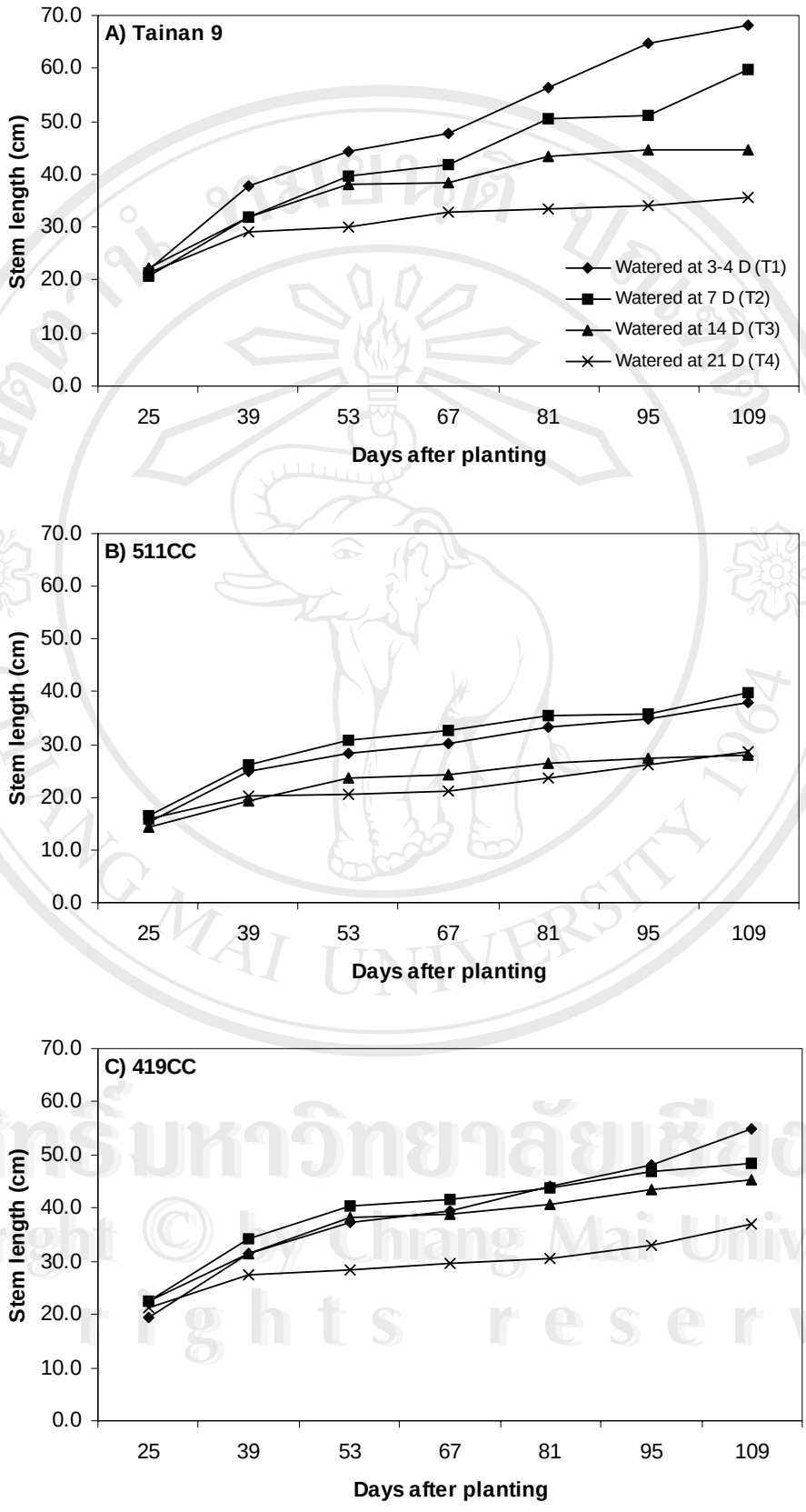

Figure 3.9 Main stem length of three peanut genotypes growing under four water treatments.

Root growth

Root growth pattern of three peanut genotypes at different soil depths (5, 25, 50 and 75 cm) observing at various time periods are shown in Figure 3.10-3.13. The plants receiving T1, T2 water treatment trended to produce more root length at the two upper soil depths (5 and 25 cm) and root length decreased with increasing soil depth below. While more root length was observed at the two lower soil depths (50 and 75 cm) for T3 and T4 plant, and the length of root trended to increase with increasing soil depth.

At 5 cm soil depth, root length did not differ significantly among peanut genotypes at any observation time. Water treatments significantly affected root growth at 93 DAP. Plants receiving T2 treatment had greatest root length, which was 6% greater than T1 treatment, 63% greater than T3 treatment and 77% greater than T4 treatment.

At 25 cm soil depth, there were significant effects of water treatment at 79 and 93 DAP. No significant effect of water treatment was found at any plant age. However, significant interactions between peanut genotype and water treatment were observed at 65 DAP. At 79 DAP, root length was greatest in 419CC, followed by Tainan 9 and 511CC had the least. By 93 DAP, 419CC and Tainan 9 both had 32% greater root length than 511CC.

At 50 cm soil depth, neither water treatment nor genotype had significant effect on root length any plant age. Significant interactions between water treatment and genotype were observed at 93 DAP.

At 75 cm soil depth, no statistically different root lengths were observed among water treatments, genotype, or their interactions.

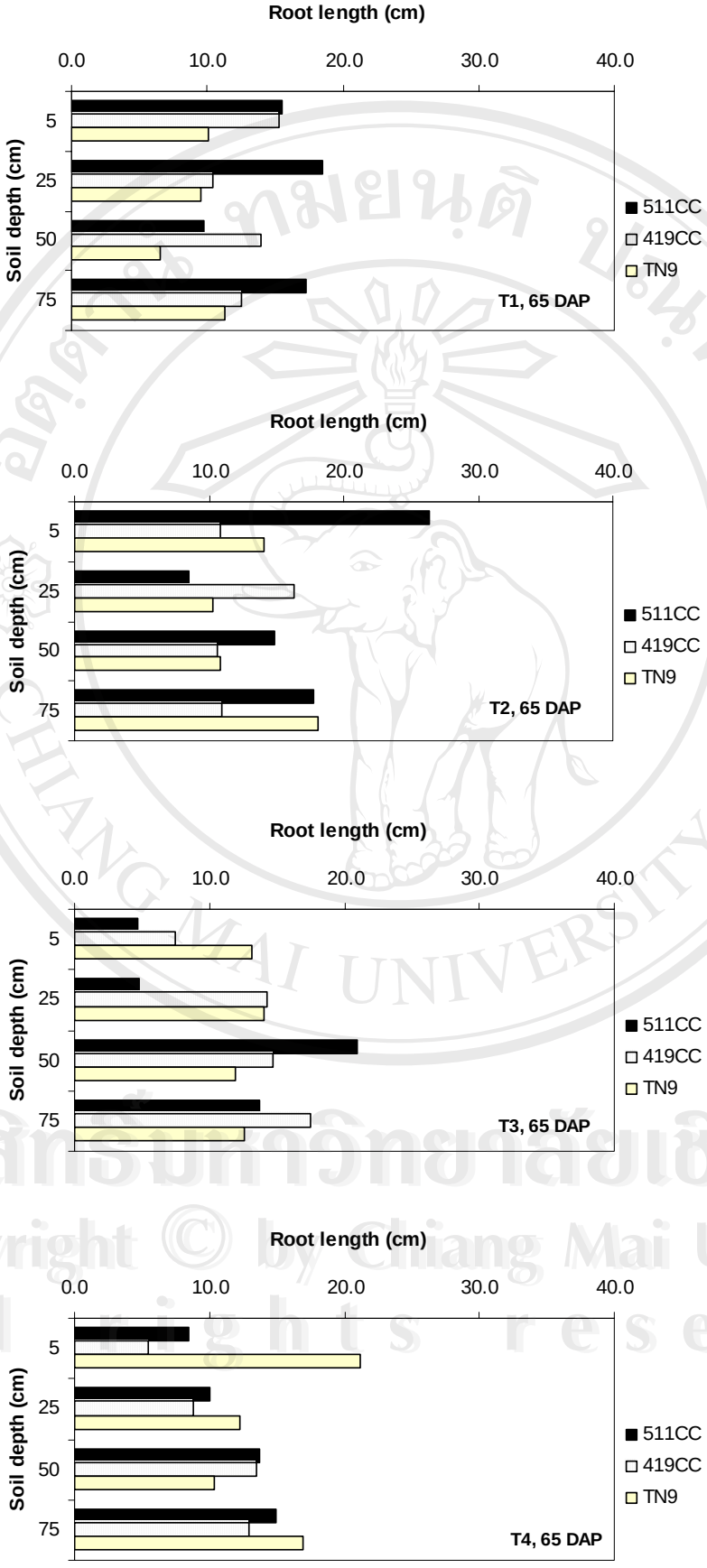

Figure 3.10 Root length at four soil depth for three peanut genotypes growing under four water regimes; observed at 93 DAP.

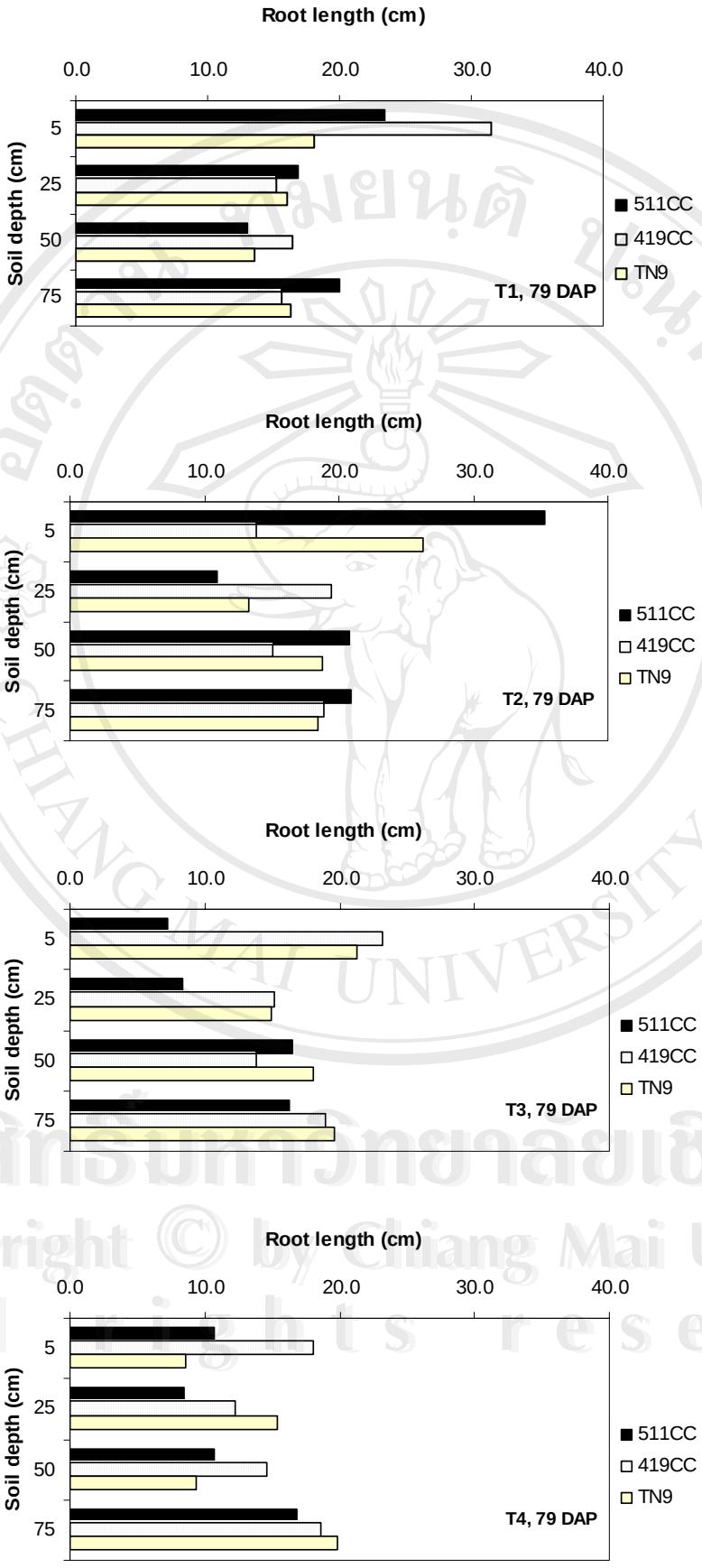

Figure 3.11 Root length at four soil depth for three peanut genotypes growing under four water regimes; observed at 79 DAP.

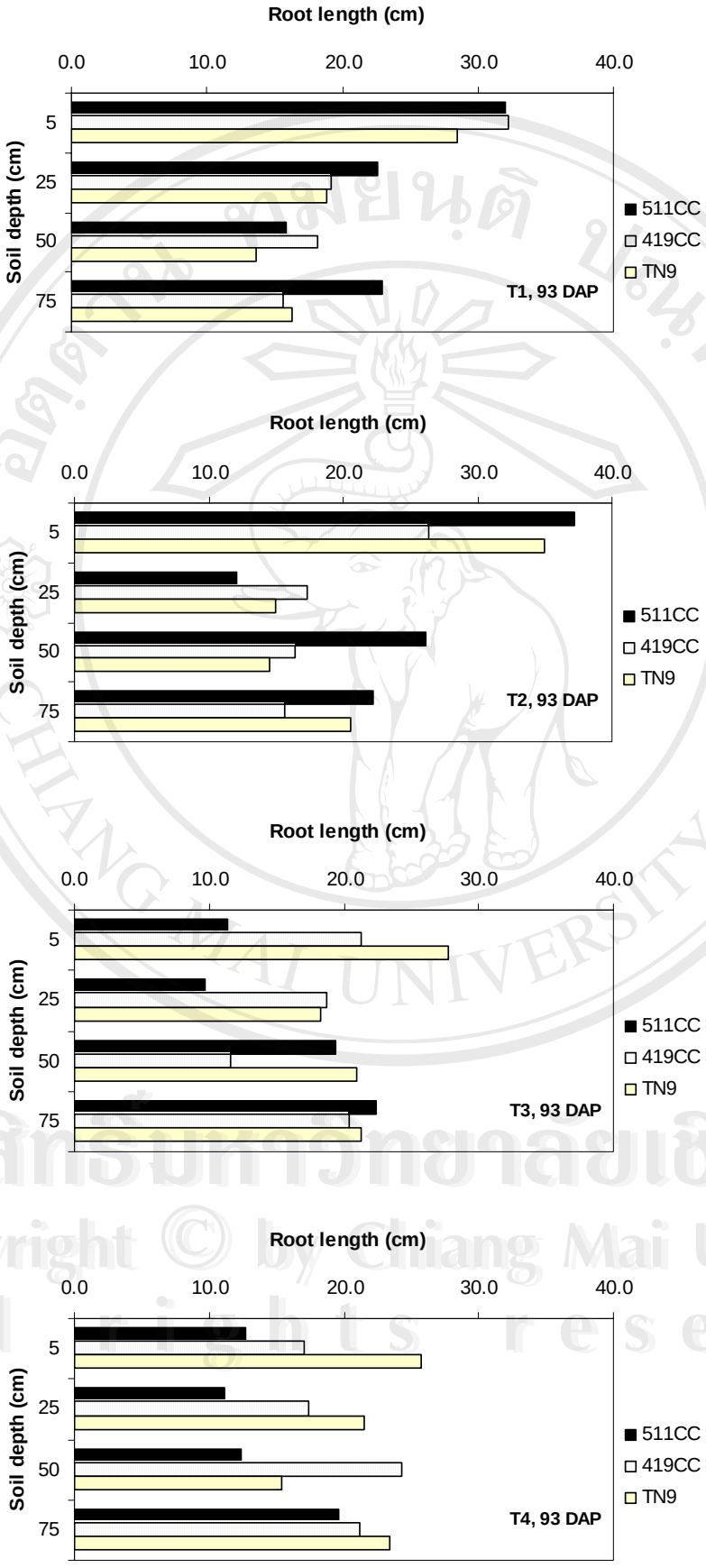

Figure 3.12 Root length at four soil depth for three peanut genotypes growing under four water regimes; observed at 93 DAP.

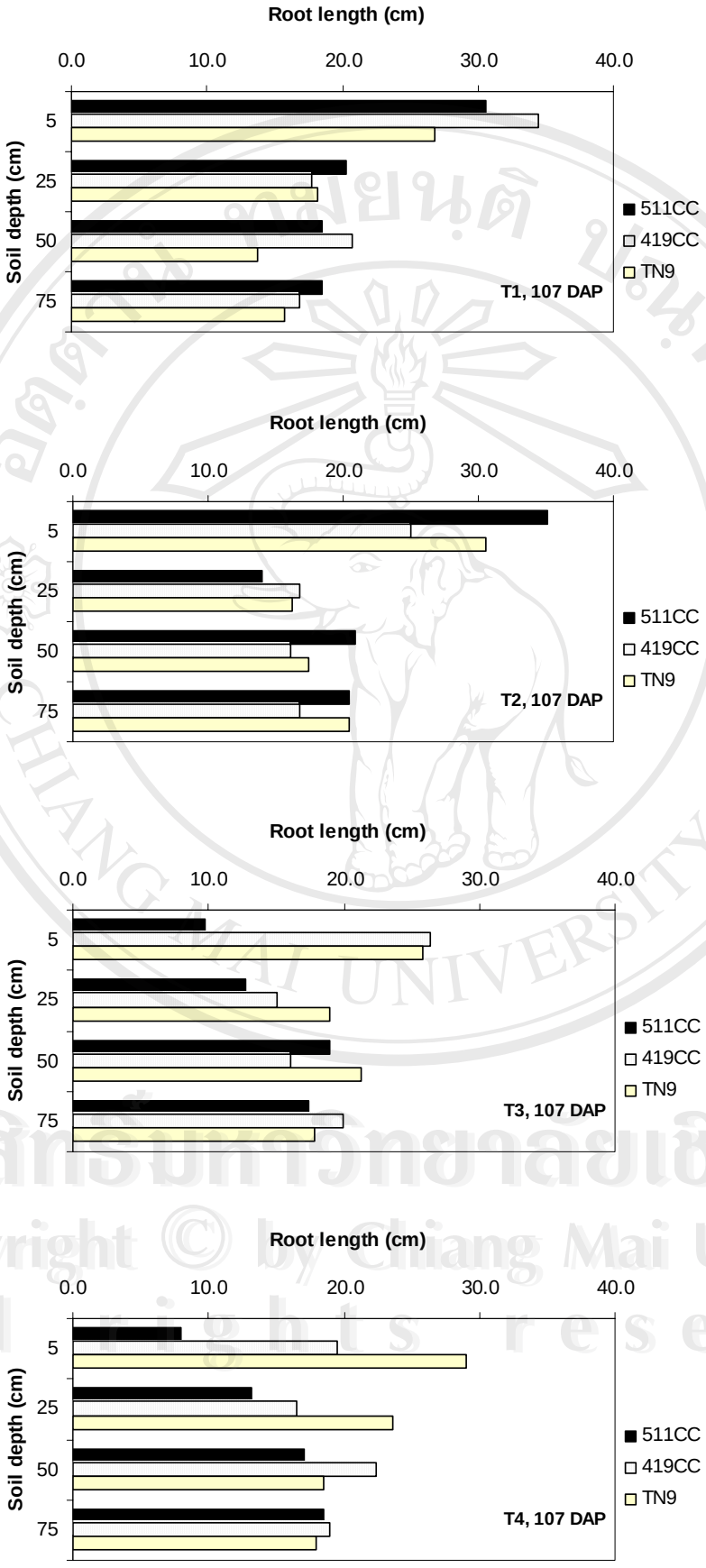

Figure 3.13 Root length at four soil depth for three peanut genotypes growing under four water regimes; observed at 107 DAP.

Final Sampling

Plant traits of peanut genotypes at the final sampling are shown in Table 3.2-3.5. There were significant effects of genotype and water treatment on leaf area, specific leaf area (SLA), shoot dry weight, and pod dry weight.

Area of 100 leaflets was greatest in Tainan 9 (348.3 cm^2) followed by 419CC (300.7 cm^2) and 511CC (179.6 cm^2). Among water treatments, leaf area was largest in T1 and T2 (averaged 309.7 cm^2), followed by T3 (268.2 cm^2) and T4 (219.2 cm^2). Leaf area reductions in T4 plants were 20% for 511CC, 32% for 419CC, and 31% for Tainan 9 (Table 3.2).

Mean SLA for 511CC was 48% greater than that of 419CC and 50% greater than Tainan 9. Comparing water treatments, the highest SLA was found in T1 no significant differences among the other three water treatments. The reduction in SLA of T4 plants was 40% for 511CC, 27% for 419CC and 13% for Tainan 9 as compared with T1 treatment (Table 3.3).

Shoot dry weight significantly differed among genotypes and among water treatments. Genotype 511CC had 27% greater shoot dry weight than Tainan 9 and 35% greater than 419CC. Shoot dry weight was reduced by 28% in T3 plants and by 47% in T4 plants (Table 3.4). Comparing among water treatments of each genotype showed that in 511CC, shoot dry weight was reduced by 52% in T3 and by 53% in T4. In Tainan 9, shoot dry weight was decreased by 33% in T3 and 55% in T4 as compared with T1 treatment. In 419CC, shoot dry weight was reduced by 29% in T3 and 37% in T4 as compared with T2 treatment.

There were significant effects of genotype and water treatment on pod dry weight $plant^{-1}$ (Table 3.5). Among genotypes, the greatest pod dry weight was found

in Tainan 9 (171.1 g) followed by 419CC (154.7 g) and 511CC (138.8 g). Pod dry weight was greater in T1 and T2 treatments of all genotypes. In contrast, pod dry weight was least in T4 treatment for all genotypes. The reduction in pod dry weight was greatest in 511CC (64% at T3, 85% at T4) followed by 419CC (32% at T3, 57% at T4), and Tainan 9 (28% at T3, 53% at T4).

Table 3.2 Area of 100 leaves plant^{-1} measured at 120 DAP of 3 peanut genotypes grown under 4 water treatments at a green house of the Georgia Envirotron, GA, 2002.

Water treatment	LA (100 leaflet plant^{-1})			Mean
	511CC	419CC	Tainan 9	
	--------cm^2--------			
Every 3-4 d	180.7	392.0	379.3	317.3 a
Every 7 d	209.5	294.9	395.8	300.1 a
Every 14 d	171.8	283.9	349.0	268.2 b
Every 21 d	156.5	232.0	268.9	219.2 c
Mean	179.6 c	300.7 b	348.3 a	276.2

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

Table 3.3 Specific leaf area of 100 leaves plant^{-1} measured at 120 DAP of 3 peanut genotypes grown under 4 water treatments at a green house of the Georgia Envirotron, GA, 2002.

Water treatment	SLA (100 leaflet plant^{-1})			Mean
	511CC	419CC	Tainan 9	
	--------cm^2 g^{-1}--------			
Every 3-4 d	893.6	456.3	325.7	575.2 a
Every 7 d	464.6	387.4	316.1	389.4 b
Every 14 d	586.9	338.4	281.8	404.4 b
Every 21 d	538.5	332.8	309.2	393.5 b
Mean	620.9 a	378.7 b	320.7 c	440.6

Table 3.4 Shoot dry weight of 3 peanut genotypes grown under 4 water treatments in a green house of the Georgia Envirotron, GA, 2002.

Water treatment	Shoot dry weight			Mean
	511CC	419CC	Tainan 9	
	-------- g plant^{-1}--------			
Every 3-4 d	585.4	264.4	379.7	409.8 a
Every 7 d	444.7	328.7	364.3	379.2 a
Every 14 d	279.3	231.9	246.2	252.5 b
Every 21 d	273.7	208.6	170.9	217.7 b
Mean	395.8 a	258.4 b	290.3 b	314.8

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

Table 3.5 Dry weight of mature peanut pods grown under 4 water treatments in a green house of the Georgia Envirotron, GA, 2002.

Water treatment	Pod dry weight			Mean
	511CC	419CC	Tainan 9	
	--------g plant^{-1}--------			
Every 3-4 d	227.5	169.6	216.6	204.6 a
Every 7 d	211.6	212.3	211.1	211.7 a
Every 14 d	81.0	145.4	155.0	127.1 b
Every 21 d	35.1	91.3	101.8	76.1 c
Mean	138.8 b	154.7ab	171.1 a	154.9

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

***Aspergillus flavus* infection**

Minirhizotron observations at 5 cm soil depth showed fluorescence of *A. flavus* near roots (Figure 3.14) and pods (Figure 3.15). Data of *A. flavus* colonization as analyzed by QuaCos program are shown in Table 3.6. Relative green fluorescence color value did not differ significantly among peanut genotypes or among water treatments. However, genotype 419CC had greatest density of green value (9,112) followed by Tainan 9 (8,261) and 511CC (7,303). The mean of green value increased as increasing water stress duration. It was greatest in T4 treatment (9,310) followed by T3 (8,421), T2 (8,028), and lowest in T1 (7,143).

Effect of water treatments and genotype on *A. flavus* infection of pod shells and seeds are summarized in Table 3.7-3.8. There were significant effects of water treatments on both pod shell and seed infections by *A. flavus* for all genotypes. Percentages of *A. flavus* colonizations were highest in T4 treatment for pod shell, and in T2 treatment for seed. Incidence of pod shell infection increased with water stress duration. Colonization was greatest in T4 (78%) and least in T1 (49%). In contrast, seed infection was greatest in T2 (26%) and least in T1 (12%). Infections of shells and seeds did not significantly differ among genotypes. However, incidence of fungal colonization in both shell and seed were relatively higher in 511CC than other two genotypes. There was a significant correlation between pod shell and seed infection ($r = 0.619^*$).

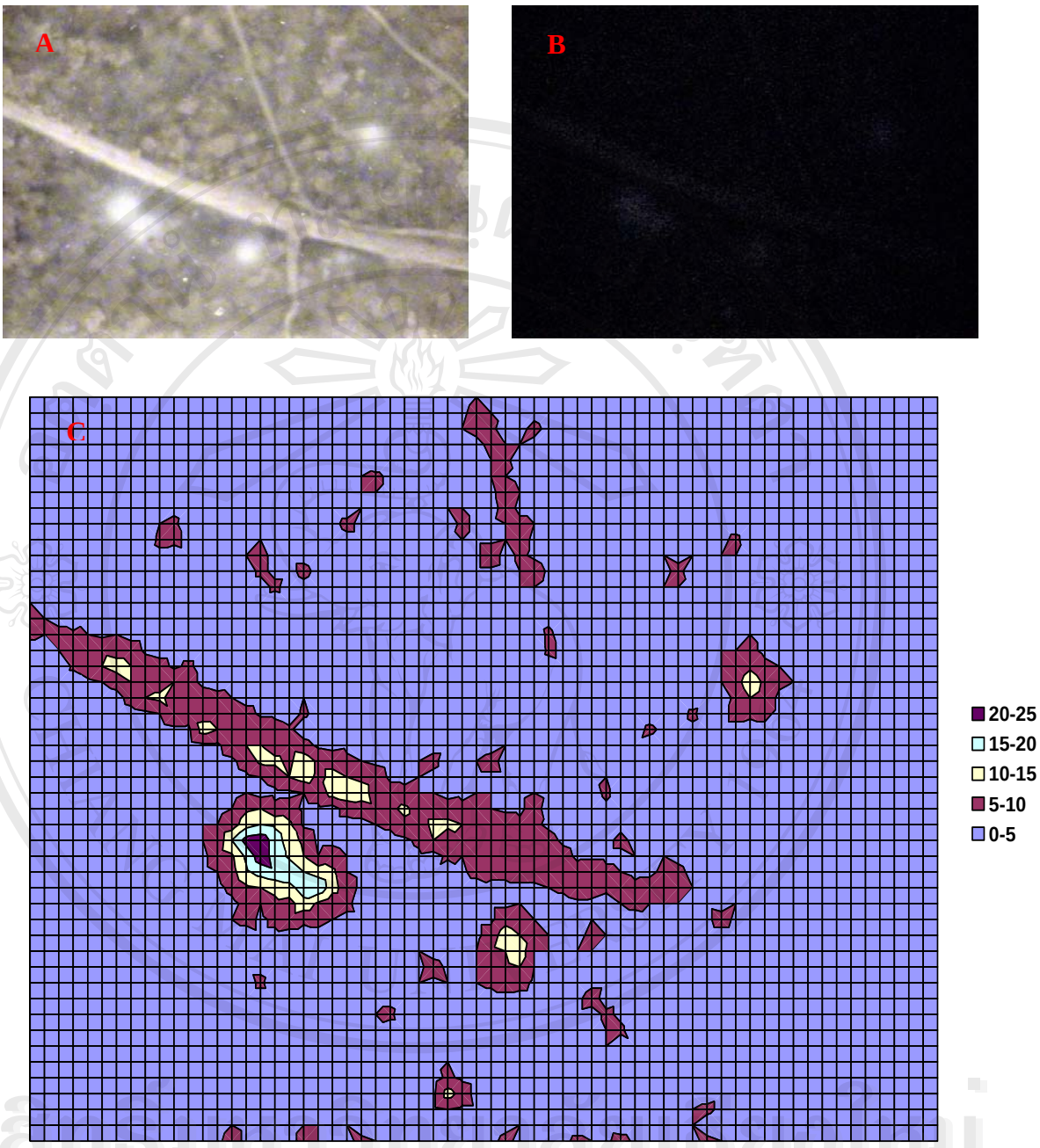

Figure 3.14 Root image as observed by a minirhizotron camera: (A) observed with a white light and (B) observed with UV light. (C) *Aspergillus flavus* population density on roots as estimated by QuaCos.

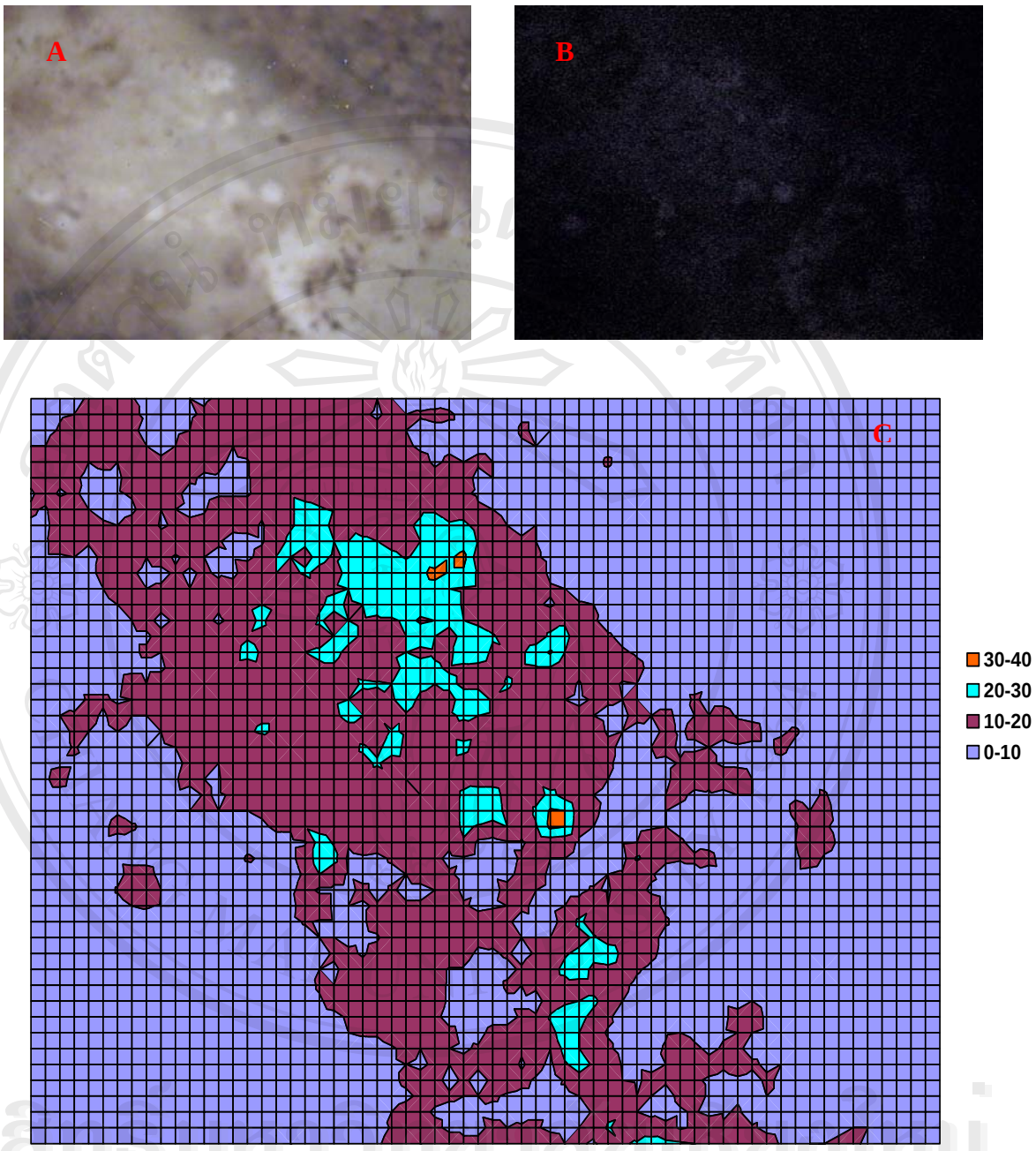

Figure 3.15 Pod image as observed by a minirhizotron camera: (A) observed with a white light and (B) observed with UV light. (C) *Aspergillus flavus* population density on pod as estimated by QuaCos.

Table 3.6 Relative *Aspergillus flavus* population density (64 × 48 groups of each image) on the root zone at 5 cm depth soil layer of three peanut genotypes grown under 4 water regimes at the Georgia Envirotron, GA, 2002.

Genotype	Water treatment				Mean
	Every 3-4 D	Every 7 D	Every 14 D	Every 21 D	
	Relative green fluorescence color value, image total				
419CC	7,723	10,372	8,712	9,640	9,112
Tainan 9	5,964	6,873	9,596	10,613	8,261
511CC	7,741	6,840	6,954	7,678	7,303
Mean	7,143	8,028	8,421	9,310	8,225

Table 3.7 Peanut pod shell infection by *Aspergillus flavus* grown under 4 different water regimes at a green house of the Georgia Envirotron.

Genotype	Water treatment				Mean
	Every 3-4 D	Every 7 D	Every 14 D	Every 21 D	
	A. flavus infection, %				
511CC	68.8	58.3	85.4	64.6	69.3 ns
419CC	25.0	89.6	64.6	87.5	66.7
Tainan 9	54.2	64.6	75.0	81.3	68.8
Mean	49.3 b	70.8 a*	75.0 a	77.8 a	68.3

* Means followed by the same letter are not significantly different at P = 0.05 by LSD. LSD (0.05) genotype = 19.63, LSD (0.05) water treatment = 22.67.

Table 3.8 Peanut seed infection by *Aspergillus flavus* grown under 4 different water regimes at a green house of the Georgia Envirotron.

Genotype	Water treatment				Mean
	Every 3-4 D	Every 7 D	Every 14 D	Every 21 D	
	A. flavus infection, %				
511CC	16.7	20.8	45.8	12.5	24.0 ns
419CC	12.5	37.5	6.3	18.8	18.8
Tainan 9	6.3	20.8	16.7	27.0	17.7
Mean	11.8 b	26.4 a*	22.9 ab	19.4 ab	20.1

* Means followed by the same letter are not significantly different at P = 0.05 by LSD. LSD (0.05) genotype (G) = 12.59, LSD (0.05) water treatment (W) = 14.54, LSD (0.05) G × W = 12.80.

Biochemical responses of peanut pods to drought and *Aspergillus flavus* infection

Pod shell and seed infection by *Aspergillus flavus*

Application of cracked corn inoculum and spore suspension of *A. flavus* onto peanut plants significantly increased pod shell and seed colonization by this fungus. Infections of inoculated plants were 29.6% for pod shell and 9.6% for seed. However, the fungus also colonized some non-inoculated plants.

A significant effect of irrigation on fungus colonization was found only for pod shell (Table 3.9). Pod shell colonization increased from 15.6 to 23.7% when plants were subjected to water deficit. In other words, pod shell in water-deficit treatments had 53.0% greater fungal colonization than those in the well-watered treatment.

Among the three peanut genotypes, the highest incidence of pod shell infection was found in genotype 419CC with an infection of 23.0%, followed by Tainan 9 with 21.0% and 511CC with 16.0%.

Chemical composition in pod shell and seed coat of peanut

Analyses of variance for CT, Ca, Mg, Fe, Mn, and Zn contents are presented in Table 3.10. Condensed tannin contents in pod shell and seed coat of non-inoculated plants were higher than those in inoculated plants. In contrast, pod shell of inoculated plants had higher levels of Ca, Mg, and Fe while these two elements (except Fe) were lower in seed coat compared with non-inoculated plants (Table 3.11).

Genotype 419CC had 32.0% more CT in pod shell than Tainan 9 and 56.0% more than 511CC, whereas CT in seed coat was greatest in 511CC, which had 18.0% more seed coat CT than 419CC and 27.0% more than Tainan 9. Condensed tannin in pod shell and seed coat was greater in non-inoculated plants than in inoculated plants.

Clearly, application of fungal inoculum decreased CT accumulation in both pod shell and seed coat of peanut. Water-stressed plants had numerically higher CT content in pod shell but lower in seed coat than well-watered plants, however, they were not significant. The CT content in pod shell was significantly greater in 419CC and Tainan 9 than in 511CC. However, the amount of CT in seed coat was greaterer for 511CC than in the two genotypes, with 419CC being intermediate and Tainan 9 having the least.

Mineral composition analyses revealed that genotype Tainan 9 had a greater concentration of Ca in both pod shell and seed coat than the other two genotypes. The concentration of Ca in pod shell was 3.34 g $kg^{-1}$for Tainan 9, 3.06 g kg^{-1} for 419CC and 2.01 g kg^{-1} for 511CC whereas in seed coat, there was 3.68 g kg^{-1} for Tainan 9, 3.25 g kg^{-1} for 419CC and 2.72 g kg^{-1} for 511CC. There was a similar concentration of Mg in pod shell of Tainan 9 and 419CC with approximately 2.44 g kg^{-1} whereas genotype 511CC contained 1.85 g kg^{-1}. However, Mg content of seed coat was not different among peanut genotypes. For Mn content in seed coat, Tainan 9 and 419CC had approximately 0.015 g kg^{-1} and 0.012 g kg^{-1} for 511CC (Table 3.12). There was significant effect of inoculation on Ca, Mg, and Fe in pod shell and also on Ca, Mg, and Mn in seed coat. In this study, Ca, Mg, and Fe contents in pod shell were higher in plants that received inoculum than in those that did not. In contrast, Ca, Mg, and Mn contents in seed coat were greater in non-inoculated than in inoculated plants. Irrigation also influenced mineral composition of pod shell and seed coat. Concentrations of Ca and Fe in pod shell were significantly reduced by water deficit, with a reduction of 19.0% for Ca and 32.0% for Fe. Conversely, the concentration of Zn in seed coat increased for plant subjected to water deficit (Table 3.13).

Table 3.9 Colonization of pod shell and seed of three peanut genotypes by *Aspergillus flavus* as affected by inoculation and irrigation methods.

Genotype	Inoculated		Noninoculated		Mean
	Irrigation		Irrigation		Genotype
	WW†	WD‡	WW	WD	
Pod shell	---------- % ----------				
Tainan 9	24.4	42.2	6.7	8.9	20.6ab*
419CC	22.2	42.2	11.1	15.6	22.8a
511CC	22.2	24.4	6.7	8.9	15.6b
Mean (WW)		15.6b			
Mean (WD)		23.7a			
Mean (F)§	29.6a		9.6b		
Seed	---------- % ----------				
Tainan 9	11.1	11.1	0.0	6.7	7.2a
419CC	8.9	11.1	0.00	2.2	5.6a
511CC	6.7	8.9	2.2	2.2	5.0a
Mean (WW)		4.8a			
Mean (WD)		7.0a			
Mean (F)	9.6a		2.2b		

* Means followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

† WW = Well-watered treatment.

‡ WD = Water-deficit treatment.

§ F = Inoculation treatment; inoculated and noninoculated by *A. flavus*.

Table 3.10 Mean square error values from analyses of variance of condensed tannin (CT) and mineral element (Ca, Mg, Fe, Mn, and Zn) contents in pod shell and seed coat of three peanut genotypes as affected by inoculation and irrigation methods.

Source	d.f.	CT	Ca	Mg	Fe	Mn	Zn
Pod shell							
Fungus (*F*)	1	8.03*	4.82**	0.48*	0.4107**	0.0001	0.00008
Genotype(*G*)	2	7.07*	5.89**	2.53**	0.0283	0.0002**	0.00004
Irrigation (*I*)	1	2.15	3.09*	0.03	0.3034**	0.0001	0.00007
FxG	2	1.66	0.22	0.08	0.0161	0.0001	0.00006
FxI	1	0.07	0.01	0.08	0.0009	0.0001	0.00002
GxI	2	0.03	0.98	0.21	0.0068	0.0001	0.00001
FxGxI	2	2.08	0.08	0.01	0.0099	0.0001	0.00001
Source	d.f.	CT	Ca	Mg	Fe	Mn	Zn
Seed coat							
Fungus (*F*)	1	124656**	0.73*	1.00**	0.0003	0.0003**	0.00001
Genotype (*G*)	2	146741**	2.77**	0.14	0.0001	0.0002*	0.00001
Irrigation (*I*)	1	8974	0.05	0.08	0.0005	0.0001	0.00004*
FxG	2	36445	0.01	0.04	0.0002	0.0001	0.00004*
FxI	1	10221	2.05**	0.02	0.0001	0.0001	0.00001
GxI	2	21761	0.09	0.05	0.0002	0.0001	0.00002
FxGxI	2	30581	0.13	0.01	0.0001	0.0001	0.00001

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 3.11 Means of condensed tannin (CT) and mineral contents in pod shell and seed coat of non-inoculated and inoculated plant by *Aspergillus flavus*.

Inoculation	Mineral content (g kg^{-1})					CT
	Ca	Mg	Zn	Fe	Mn	(A_{550} g^{-1})
Pod shell						
Non-inoculated plant	2.43b*	2.06b	0.022a	0.377b	0.032a	3.81a
Inoculated plant	3.17a	2.29a	0.025a	0.591a	0.032a	2.87b
Seed coat						
Non-inoculated plant	3.36a	2.20a	0.025a	0.075a	0.017a	949.03a
Inoculated plant	3.07b	1.86b	0.025a	0.069a	0.011b	831.34b

Table 3.12 Means of condensed tannin (CT) and mineral contents in pod shell and seed coat of three peanut genotypes.

Genotype	Mineral content (g kg^{-1})					CT
	Ca	Mg	Zn	Fe	Mn	(A_{550} g^{-1})
Pod shell						
Tainan 9	3.34a*	2.46a	0.025a	0.489a	0.035a	3.17ab
419CC	3.06a	2.42a	0.022a	0.529a	0.033a	4.17a
511CC	2.01b	1.85b	0.022a	0.433a	0.027b	2.67b
Seed coat						
Tainan 9	3.68a	1.97b	0.025a	0.073a	0.015a	798.48b
419CC	3.25b	2.16a	0.025a	0.069a	0.015a	839.09b
511CC	2.72c	1.98ab	0.025a	0.072a	0.012b	1012.08a

* Means within column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Table 3.13 Means of condensed tannin (CT) and mineral contents in pod shell and seed coat of peanut as affected by well-watered and water-deficit conditions.

Irrigation	Mineral content (g kg^{-1})					CT
	Ca	Mg	Zn	Fe	Mn	(A_{550} g^{-1})
Pod shell						
Well-watered	3.09a*	2.20a	0.022a	0.576a	0.034a	3.09a
Water-deficit	2.51b	2.15a	0.025a	0.392b	0.030a	3.58a
Seed coat						
Well-watered	3.18a	2.08a	0.023b	0.068a	0.014a	905.97a
Water-deficit	3.25a	1.98a	0.026a	0.076a	0.014a	874.39a

* Means within column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Correlations between pod shell and seed infection with their chemical compositions were significant only for Mg and Mn contents. There were negative correlations among Mg and Mn contents in seed coat with pod shell infection (r = -0.639* for Mg and -0.534* for Mn, n = 12) and also between those two elements with seed infection (r = -0.799* for Mg and -0.645* for Mn, n = 12). Linear regression equations of Mg and Mn contents in seed coat of three peanut genotypes in relation to seed infection by *A. flavus* are listed in Table 3.14. For each genotype, linear relationships between Mg content and infected seed were obtained only in genotype Tainan 9 and 511CC ($r^2 > 0.90$) but not for 419CC. There were highly-positive correlations between Ca with Mg, Mn, and Fe in pod shell.

Table 3.14 Linear regression equations and coefficients between seed infection by *Aspergillus flavus* (%) and content of Mg and Mn in seed coat (g kg^{-1}) of three peanut genotypes (n = 4).

Genotype	Equation	r^2
Mg		
Tainan 9	-29.17x + 64.53	0.97*
419CC	-22.63x + 54.27	0.73
511CC	-11.79x + 28.33	0.91*
Mn		
Tainan 9	-2244.5x + 40.89	0.85
419CC	-1268.5x + 24.39	0.80
511CC	-631.65x + 12.53	0.72

Crude protein and crude fat in peanut cotyledon

Contents of crude protein and crude fat as affected by inoculation and irrigation methods are shown in Table 3.15. Cotyledons of plants subjected to water deficit had 9.0% more crude protein but had 7.0% less crude fat compared with well-watered plants. Cotyledon of non-inoculated plants contained significantly more crude protein but less crude fat than those from inoculated plants. Mean crude protein of inoculated plants was 32.0% and crude fat was 50.0%, while non-inoculated plants had crude protein of 35.0% and crude fat of 47.0%. There were significant differences among the genotypes for crude protein content, while no significant differences were found in crude fat content. Genotype Tainan 9 and 419CC contained approximately 34.0% crude protein followed by 511CC with 32.0%. Crude fat contents of all three

genotypes were similar with a mean of 49.0%. The significant interactions of Genotype (G) × Irrigation (I) and G × I × F (Fungus) were found in crude fat content. The F x I interaction was significant for crude protein.

Table 3.15 Effects of inoculation and irrigation methods on crude protein and crude fat contents in the cotyledon of three peanut genotypes.

Genotype	Inoculated		Noninoculated		Mean
	Irrigation		Irrigation		Genotype
	WW†	WD‡	WW	WD	
Crude protein	---------- % ----------				
Tainan 9	30.7	35.3	35.2	35.3	34.2a
419CC	31.4	34.0	32.8	36.3	33.6ab
511CC	28.0	33.6	33.4	33.9	32.2b
Mean (WW)		31.9b			
Mean (WD)		34.8a			
Mean (F)§	32.2b		34.5a		
Crude fat	---------- % ----------				
Tainan 9	53.9	46.8	49.1	44.9	48.7a
419CC	47.2	54.4	48.8	45.9	49.1a
511CC	52.6	45.5	49.8	44.5	48.1a
Mean (WW)		50.2a			
Mean (WD)		47.0b			
Mean (F)	50.1a		47.2b		

* Means followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

† WW = Well-watered treatment.

‡ WD = Water-deficit treatment.

§ F = Inoculation treatment; inoculated and noninoculated by *A. flavus*.

DISCUSSION

Effect of drought duration on peanut growth and *Aspergillus flavus* infection

Shoot growth

This experiment examined shoot and root growth of three peanut genotypes grown under differing four water treatments. Different water treatment had no effect on leaflet size but it had significant effect on leaflet number plant^{-1}. Number of leaflets was significantly reduced by water deficit, which it was the least in plants receiving T4 water treatment. Of all peanut genotypes, 511CC had smaller leaflets than Tainan 9 and 419CC but it had more leaflets than the others, which resulted in greatest in leaf area plant^{-1}. Water deficit significantly reduced main stem length but not branch number. In this study, T3 and T4 plants had main stem to be shorter than those receiving T1 and T2 water treatment. The reduction in main stem length caused by water deficit was greatest in Tainan 9, followed by 511CC. While in 419CC, main stem did not differ between T1, T2 and T3 treatment, however shorter main stem was observed in T4 treatment.

In general, the more drought tolerant 511CC had smallest leaflet size, more leaflets and largest in total leaf area plant^{-1}, shorter main stem, and more branches than 419CC which reported as drought intolerant and unknown drought tolerant Tainan 9 genotype.

Root growth

The root length of planting receiving T1 and T2 water treatments tended to have greater in the two upper soil layer (5 and 25 cm). While in T3 and T4 plants, the root length was larger in the deeper soil depth (50 and 75 cm). The length of root

decreased with increasing soil depth for plants receiving adequate water and tended to increase with increasing soil depth for plant suffering from water deficit. These results indicated that water stress stimulates the growth of roots into deeper soil layer. Allen *et al.* (1976) concluded from measured soil water extraction that during water stress, root in lower depths continue to grow deeper even though vegetative growth appears to stop. They further stated that peanut roots effectively extracted soil water to depths of at least 189 cm in a fine sand soil. Pateña (2000) also observed that under water deficit, peanut plants that take up water from deeper soil layers could continue growth processes longer than plants with shallow root systems. Plants that were watered weekly (T2) had the longest root at 5 cm soil depth. Genotype 511CC maintained more root length in the 5 cm and 75 cm depths than other two genotypes. This behavior would allow this genotype to deplete water from the soil profile during a period of water deficit. Devries *et al.* (1989) reported that peanut cultivar Florunner had greater root length density in deeper soil layers (60-150 cm) than soybean and cowpea during drought periods. This trait contributes to peanut's ability to avoid drought. Pandey *et al.* (1984) showed that peanut had greater root length density deeper in the soil than other legumes when grown under drought.

Final sampling

There were significant effects of water treatment and genotype on leaf area SLA, shoot dry weight, and pod dry weight. Plants receiving T3 and T4 treatments had significant less leaf area, SLA and shoot dry weight than those received T1 and T2 treatment. The reduction of leaf area due to water deficit was greater in 419CC and Tainan 9 than 511CC. Shoot dry weight was greater in 511CC than in 419CC and Tainan 9. The better rooting in upper and deeper soil depths for 511CC and more

leaflets had resulted in better shoot dry weight than the other two genotypes. However, genotype 419CC appeared to have less effect from water deficit, which shoot dry weight reduction was the least.

Adequate pod zone moisture is critical for development of pegs into pods. Wright *et al.* (1994) reported that pod formation is affected by a dry pod zone and Sexton *et al.* (1997) has reported that peanut fruit growth is sensitive to surface soil (0-5 cm) conditions due to its subterranean fruiting habit. Dry pegging zone soil delayed pod and seed development. In this study, 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. Reduction in pod dry weight after prolonged drought has been reported by Meisner and Karnok (1992), who found that pod dry weights were significantly reduced by a 30-day water stress during pod development Water deficits during seed development reduce pod and seed weights. Genotype 511CC has been classified as drought resistant by Holbrook *et al.* (1993). In this study 511CC trended to have better shoot and root growth when exposed to water deficit than 419CC and Tainan 9 before harvest. However, water deficit reduced pod dry weight of 511CC more than it reduced that of the other genotypes. This reduction of pod dry weight in 511CC may have resulted from white fly damage during pod and seed development and rodent damage also observed at pod maturity, both of which lead to fewer pods plant^{-1} in 511CC. Moreover, this genotypes has growth habit as bunch type, thus the container surface area may limited the space available for peg penetrations in this green house experiment. Restricted soil surface area may have been more disadvantageous for 511CC than other two genotypes.

***Aspergillus flavus* infection**

The minirhizotron observation and subsequent QuaCos analysis, allowed me to detect the fluorescence of GFP *A. flavus* population around the roots and pods zones. Water deficit increased *A. flavus* population density around the root and pod zones of all genotypes. It is possible that roots or pods of water deficit plants may slough more cells and exude more soluble substrates than plants receiving adequate water. These substrates might include glucose (Pass and Griffin, 1972), sucrose, and fructose (Hale and Griffin, 1976), thereby allowing greater *A. flavus growth* and development. Although significant differences in *A. flavus* population densities were not found among three peanut genotypes, drought-susceptible 419CC had the largest green fluorescing values and 511CC the smallest. These results agreed with Ingram *et al.* (1999), who reported that *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.

Incidence of *A. flavus* infections on both pod shell and seed were significantly affected by water treatment. Fungal colonization was relatively high in plants receiving T4 and T2 water treatments in both pod shell and seed. Recovery of *A. flavus* from pod shells increased with increasing water deficit duration. Azaizeh *et al.* (1989) reported that low soil moisture under both long- and short-duration droughts enhanced colonization of peanut pod shell and seed when compared to non-stressed conditions. Contrary to expectation, plants receiving adequate water (T2) had as high incidence of *A. flavus* infection as those subjected to water deficit (T3 and T4).

Pod shells of all genotypes contained relatively high levels of *A. flavus* colonization (means 67-70%), whereas little seed infection was observed. Genotype

511CC, which is classified as aflatoxin resistant, appeared to have greater colonization of both pod shell and seed than the other two genotypes, even though it had the smallest *A. flavus* population density in the soil. These results suggest that even 511CC is resistant to aflatoxin, it tolerates relatively high infection levels. Moreover the presence of fungi does not necessarily result in aflatoxin contamination (Will *et al.*, 1994). In addition, because 511CC had more incidence of rodent damage than 419CC and Tainan 9, it is possible of *A. flavus* infected on the injured shell surface before culturing on selective medium.

Biochemical responses of peanut pods to drought and *Aspergillus flavus* infection

Pod shell and seed infection by *Aspergillus flavus*

Application of cracked corn inoculum and spore suspension of *A. flavus* into peanut plants increased pod shell and seed colonization by this fungus. However, the presence of this fungus colonization was also found in non-inoculated plants, although it was small. This result may occur due to the cross contamination among treatments, because it was investigated in an opened field, in this type of study where a large inoculum of *A. flavus* is added to soil and also sprayed through the plant, it is difficult to completely prevent movement of fungus into non-inoculated plant when they are in close proximity to each other.

Irrigation effects

Colonization of pod shell and seed was greater in peanut grown under water deficit compared with well water conditions. It has been reported that invasion of *Aspergillus* spp. occurs primarily under drought conditions and is also associated with high soil temperatures (Dornor *et al.*, 1989). Increased duration of water deficit and

high soil temperature stresses generally increased peanut colonized by *A. flavus* (Sander *et al*., 1985). A late season drought reduced metabolic activity due to decline in pod water content, which increases susceptibility of peanut to fungal invasion (Cole *et al*., 1993). They also found that decreased physiological activity associated with low moisture in the soil appears to favor infestation of seeds and pods by *A. flavus*.

Genotype differences

Significant differences observed in the colonization of *A. flavus* for different genotypes indicates variability in pod shell and seed susceptibility to fungal infection. The smallest incidences of shell and seed colonization by this fungus were found in 511CC and the greatest in 419CC. Genotype 511CC, which was classified as drought and aflatoxin resistant (Holbrook *et al.*, 1993), appeared to have more resistance to fungus infection than the other two genotypes.

Chemical composition effects

Condensed tannins in pod shell and seed coat of non-inoculated plants were greater than in inoculated plants. Clearly, fungal inoculum decreased CT accumulation in both pod shell and seed coat of peanut. Water deficit plants had greater CT content in shells but less CT in seed coats than well-watered plants, however, they were not significant. Kouki and Manetas (2002) found that condensed tannin (CT) and gallotannins extracted from young leaves of *Ceratonia siliqua* decreased under water deficit. Therefore, these results agreed with them only content of CT in pod shell but not for seed coat. In the present study, CT contents in pod shell were significantly greater in 419CC and Tainan 9 than in 511CC. However, the amount of CT in seed coat was greater for 511CC than in the two genotypes with 419CC being intermediate and Tainan 9 having the least. Considering that tannins,

with known antifungal activity, are important component of legume seed coat and are partially responsible for seed coat color (Cabrera and Martin, 1989), the finding of higher CT content in seed coat than in pod shell in this study should lead to the inference that CT content of seed coat is more important in preventing fungal infection than the presence of this compound in pod shell. This hypothesis is supported by the observations that 511CC contained the most CT in seed coat (Appendix F) and also had the lowest incidence of fungus colonization. Thus, 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 to aflatoxin. Studies of the relationship between tannins found in the seed coat and infection of peanut by aflatoxigenic *Aspergillus* spp. (Azaizeh *et al.*, 1990; Sanders *et al.*, 1981) revealed that tannins extracted from mature seed coat inhibited fungal growth *in vitro* and the inhibition was concentration-dependent. In contrast, our study found no correlation between pod shell and seed infection with their CT content. However, if the data of non-inoculated plants are removed from this analysis, a negative correlation was found between seed infection and CT content in seed coat (r = -0.723, n = 6).

There was significant effect of inoculation on Ca, Mg, and Fe in pod shell and also Ca, Mg, and Mn in seed coat. In this study, Ca, Mg, and Fe contents in the pod shell were higher in plants that received inoculum than in those that did not. In contrast, Ca, Mg, and Mn contents in seed coat were greater in non-inoculated plants. Irrigation also influenced mineral compositions in shell and seed coat. Water deficit reduced most of mineral concentrations in the shell but trended to enhance mineral concentrations in seed coat. However, the significant was found only in Ca and Fe in

shell and only Zn in seed coat. These evidences may be resulted from the limitation of mineral uptake from the soil solution by water stress. Kvien *et al.* (1988) reported that seed Ca concentration of peanut decreased when plant exposed to drought during 80-110 DAP. Genotype Tainan 9 had greater content of Ca in both pod shell and seed coat than other two genotypes. Both Tainan 9 and 419CC had greater levels of Mg concentration in pod shell and Mn concentration in seed coat than 511CC. A significant correlation was observed between contents of Mg and Mn with pod shell and seed colonization. Pod shell and seed colonization by *A. flavus* were negatively related to Mg and Mn contents. For each genotype, linear relationships between Mg content and percent infected seed were obtained only in genotype Tainan 9 and 511CC ($r^2 > 0.90$) but not for 419CC. Susceptibility and prevalence of many plant diseases are associated with plant nutrition. Bledsoe *et al.* (1946) found that severity of leaf spot in peanut, caused by *Mycosphaerella arachidicola* W. A. Jenk, was associated with Mg deficiency. Luchese and Harrigan (1993) found that Mn in growing medium of *A. flavus* inhibited aflatoxin production. Thus, compositions of Mg and Mn in pod shell or seed coat are possible deterrents to fungal invasion of peanut seeds. Even though Ca contents were not significantly correlated with *A. flavus* infection, there were highly-positive correlations between Ca with Mg, Mn, and Fe in pod shell.

Crude protein and crude fat in peanut cotyledon

Cotyledons of plants subjected to water deficit had more crude protein but less crude fat than those in well-watered treatments. Moreover, cotyledons of non-inoculated plants contained significantly more crude protein but less crude fat than those of inoculated plants. There were significant differences among the genotypes for

crude protein content, while no significant differences were found in crude fat content. Dawivedi *et al.* (1996) observed that end-of-season drought (80 DAP until harvest) significantly reduced total oil and increased total protein content in peanut. Our results agree with Dawivedi *et al.* (1996), but disagree with Conkerton *et al.* (1989) and Musingo *et al.* (1989) who reported that drought had no effect on total oil and total protein content. Differences in water deficit effects on fat and protein contents could be the result of differences in genotypes and intensities of drought among these studies. Furthermore, growing condition and crop management practices also influence total oil, total protein, and fatty acid in peanut (Dwivedi *et al.*, 1993). In this study, we inoculated *A. flavus* inoculum into the soil and also sprayed through the plants. Fungal inoculation may have changed the chemical composition of peanut seed. Significant interactions of genotype (G) × irrigation (I) and G × I × F (fungus) were found in crude fat content. The F× I interaction was significant for crude protein. Protein, lipid, free and total amino acid, and free fatty acid are reported to change significantly when peanut seeds are infected with *A. flavus* or *A. parasiticus* (Deshpande and Pancholy, 1979). Lalithakumani *et al.* (1971) also reported that *A. flavus*, *Botryodiplodia* spp. and *Cladosporium herbarum* reduced oil content in peanut seed.

In conclusion, 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 composition of peanut seed by reducing most of mineral compositions (except for Zn) and also condensed tannin content of peanut pod which may enhance susceptibility of peanut pod to fungal invasion. Crude protein increased but crude fat decreased when plants were subjected to drought. Seed coats

of 511CC contained more condensed tannin, with known antifungal activity, 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.