LITERATURE REVIEW

Peanut or Groundnut

Peanut or groundnut (*Arachis hypogaea* L.) is a member of family Leguminosae. It is an annual herb of indeterminate growth habit which has been divided into the two subspecies *hypogaea* and *fastigiata*. Cultivated peanut is an allotetraploid (2n = 4x = 40) species (Stalker, 1997).

The peanut seed consists of two large cotyledons, a stem axis with leaf primordial and hypocotyl, and primary root. All primordial leaves and above-ground structures appear within the first few weeks after germination. Germination is epigeal. Leaves are alternate and pinnate with four leaflets. The main stem can be erect or prostrate and ranges from 12 to 65 cm in length. The primary root system is tap rooted but many lateral roots appear beginning 3 days after emergence (Figure 1.1 A). Flowers are borne in the axils of leaves, usually with three flowers per inflorescence. The flower is showy, ranging from light yellow to deep orange color and contains five petals: a standard, two wings, and two petals fused to form a keel. There are two calyx lobes, an awnlike one opposite the keel and a broad one opposite the back of the standard. Each flower has 10 stamens, two of which are usually not fully developed. The pistil consists of an ovary, style, and stigma (Figure 1.1 B). Anthesis and pollination usually occur at sunrise with self-pollination taking place within the closed keel of the flower, which withers and drops within 24 hours after anthesis. Pods are elongated spheres having variable amounts of surface reticulation and constriction between seeds. Pods typically contain 2-5 seeds. Seed may be round or elliptical and vary in seed coat color from off-white to deep purple and may be solid or mottled.

Seed size ranges from about 0.15 to more than 1.3 g seed^{-1} in *A. hypogaea* (Singh and Simpson, 1994), but seed as small as 0.047 g seed^{-1} are produced by wild species. Seeds in Spanish-type cultivars usually mature within 90-120 days after planting, whereas most Virginia-type cultivars take 130 days or more to mature. Peanut seeds are rich sources of edible oils, containing 40 to 50% fat, 20 to 50% protein, and 10 to 20% carbohydrate. The seeds are nutritious and contain vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine, and potassium. Peanut, peanut oil, and peanut protein meals constitute an important segment of world trade in oilseeds and products.

Suitable regions for peanut growth are from 35^{o} S to 45^{o} N latitude. It can adapt to a wide range of environments. The optimum temperature for peanut growth is between 25 and 35^{o}C; temperatures 20^{o}C retard development. A total seasonal rainfall of 500-1000 mm is generally sufficient for commercial peanut production, but a crop can be produced on as little as 300-400 mm. Soil should be light-colored, light textured with good drainage, friable, sandy loam, well supplied with calcium, and moderate amount of organic matter. Peanut grows best in a slightly acid soil with a pH of 6.0 to 6.5 Peanut tolerates soil pH from 5.5 to 7.0 but pH of 5.0 or less severely limits growth (Stalker, 1997).

To achieve maximum economic yield, competing weeds must be eliminated. An unusual nutritional requirement of peanut is calcium in the pod zone, which is an insufficient result in empty pods. The most serious fungal diseases of foliage are leaf spot and rust, which can cause significant yield loss, particularly during the wet season. The important soil borne fungus *Aspergillus flavus* and related species are widespread in peanut producing regions and infected peanut can be contaminated with

carcinogenic aflatoxins (Weiss, 2000). However, future efforts will be needed to solve several production problems, especially those related to diseases, aflatoxin, and seed quality.

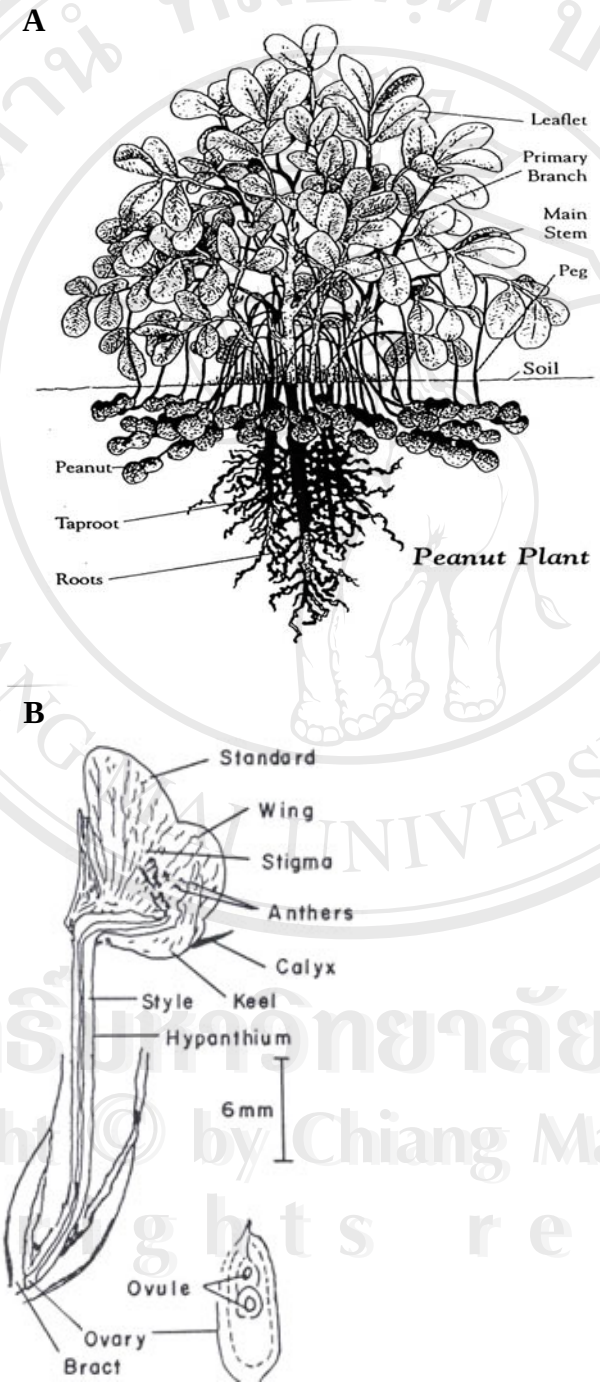

Figure 1.1 Peanut plant (A) and peanut flower components (B).

The cultivated peanut originated in South America (Bolivia and adjoining countries) and is now cultivated around the world in tropical, sub-tropical, and warm temperate climates. The countries with the largest production of peanut are India, China, U.S.A., West and Southern Africa, and Brazil. Peanut is currently produced on approximately 37 million metric ton worldwide (FAO, 2003). About 13.5 million hectare (ha) are grown in Asia, 5.3 million ha in Africa, 1.2 million ha in the Americas, and 0.1 million ha in other parts of the world (Stalker, 1997). Figure 1.2 shows the peanut production trend in the world since 1961.

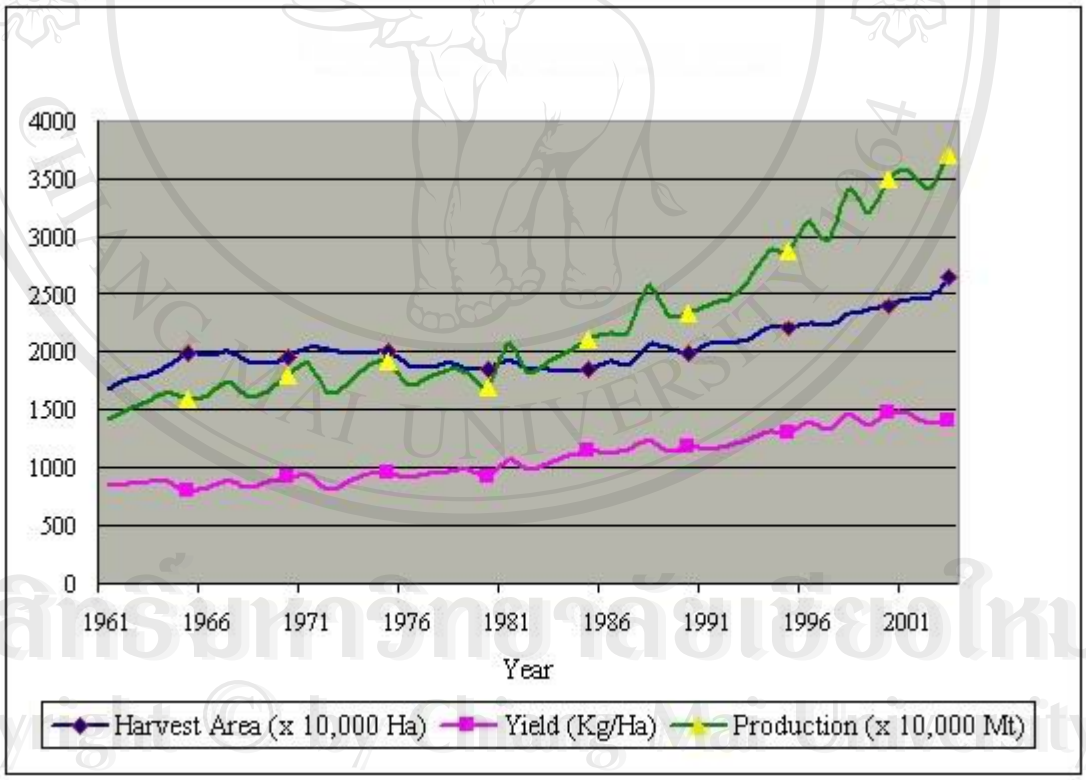

Figure 1.2 Peanut harvest area, yield, and production trend in the world since 1961 to 2001. (Source: FAO, 2003)

Peanut is believed to have been introduced to Thailand by a European trader during the Ayuthya period in the 17th century. It is an important legume and oil crop, which grown mostly by small farmers, providing significant source of cash income and important source of protein. Peanut is consumed in various forms: as an ingredient to a variety of dishes, as boiled, roasted peanut, and in confectioneries (Patanothai, 1995). The major Thai peanut growing regions are found in the North and Central Plains, and the Northeast. The Northern region had 28.7% of cultivated land under peanut, whereas the Northeast had 7.93% of cultivated land under peanut in 1995 (Sukharomana and Dobkuntod, 2003). The trend of peanut production in terms of area under cultivation, total production, and yield is given in Figure 1.3.

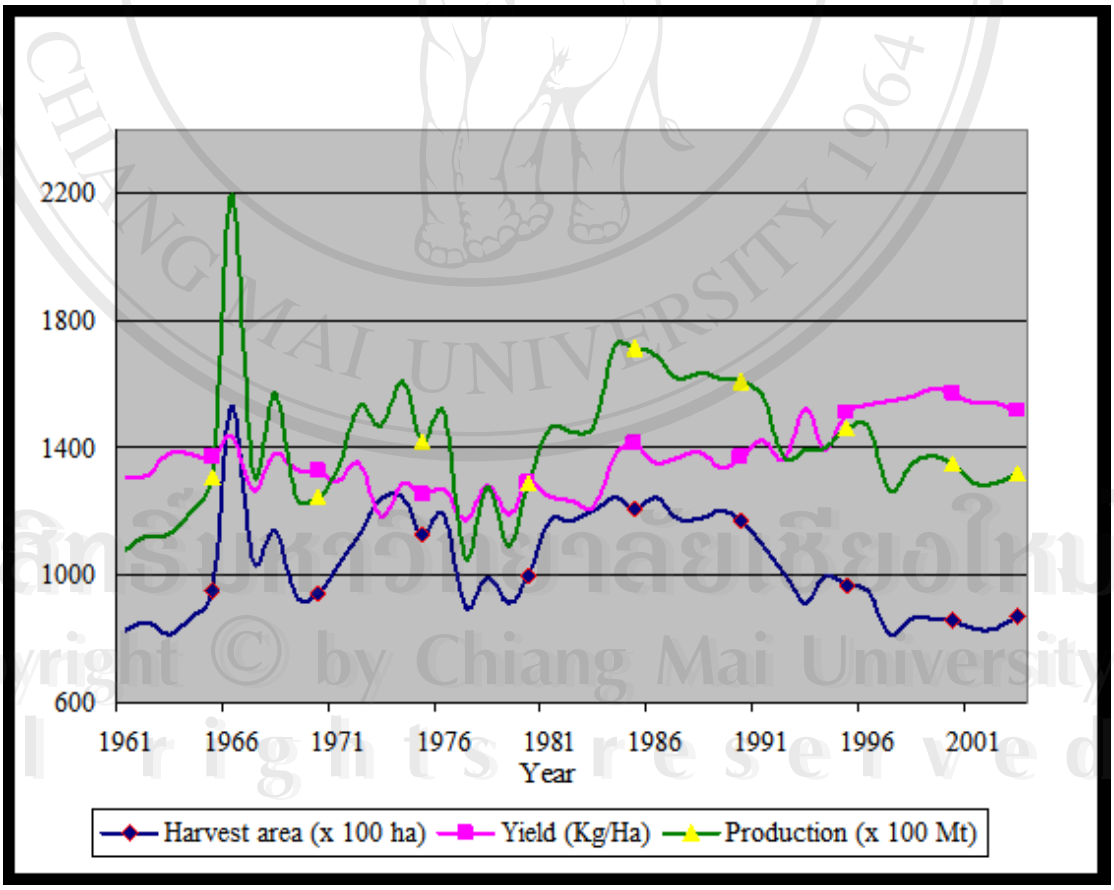

Figure 1.3 Peanut harvest area, yield, and production trend in Thailand since 1961 to 2001. (Source: FAO, 2003).

Peanut growth and development are integrated responses to many environmental, aerial, and soil factors. These factors interact, often in a complex fashion. There is much information on the methods or technique to determine the effect of genotypes, environmental factors, drought, soil, and other factors on the physiological responses of the above ground plant parts, but limited information is available on their effects on the underground plant parts. Three tools were found that could be used to determine how peanut root and pod systems respond to environmental factors, namely minirhizotron, WinRHIZO software, and QuaCos programs.

Minirhizotrons

The minirhizotron technique is a non destructive method of observing roots at various soil depths and locations throughout the plant growth duration. This method is appropriate for the study on temporal and spatial root responses to water deficit.

Minirhizotrons are clear plastic tubes, which are usually made of acrylic or cellulose acetate butyrate. Tubes are installed under the soil surface and allow periodic observations of roots through the use of a digital camera. Because geotropism directs root growth downward, vertical tube installation increases probability of roots following the soil tube interface for extended distances after roots intersect the tube, and vertical tube installation also concentrates root growth on soil-tube interface. Horizontal minirhizotron tube orientation would avoid overestimation of roots at soil-tube interface as compared with other tube orientations. Horizontal

tube orientation can easily be done in a greenhouse experiments that large containers for growing plants.

The soil-tube interface may also affect root growth and density if light penetrates through the minirhizotron tube (Klepper and Kaspar, 1994; Box, 1996), thus ends of tubes must be well sealed to prevent light from entering the tube, and inspection for light leaks is important after observing roots.

New models of minirhizotron cameras (Bartz Technology Corp., Santa Barbara, CA) allow capture and storage of root images directly into a laptop computer. Root measurements can be done from digital images using imaging software (Smucker *et al.*, 1987). Digital image measurements using software packages may provide faster and more accurate root measurements. Pateña and Ingram (2000) developed system to measure root distribution in peanut. Digital images were recorded from a minirhizotron camera directly to laptop computer hard drive instead of using videotape. This reduced image acquisition time by 36% and eliminated problems associated with manipulating video players. They concluded that this system is fast, simple and accurate for capturing minirhizotron images through low-cost software upgrade of existing minirhizotron equipment and processing digital images using low cost software.

Estimation of root length density assumes that minirhizotron cameras view roots in a soil layer 1 to 3 mm deep adjacent to the minirhizotron tube, with the actual depth of the soil layer observed depending on soil texture (Upchurch, 1987). Soil volume is estimated by multiplying the surface area observed by the minirhizotron camera by the assumed depth of view into the soil. Root length density is calculated from measured root length divided by soil volume.

Minirhizotrons have been used to study the crop root systems by many researchers (Ferguson and Smucker, 1989; Nickel *et al.*, 1995; Schröder *et al.*, 1996, Pateña and Ingram, 2000; Liedgens and Richner, 2001). This system also could be used to observe *A. flavus* growth on peanut roots and pods *in situ* with peanut plant grown in containers were inoculated with a strain of GFP *A. flavus* (Ingram *et al.*, 1999; Pateña, 2000).

Root analysis software

WinRhizo

WinRhizo (Regent Instruments, Inc., Quebec, Canada) is a commercial image analysis system specifically designed for root measurements. This system atomically analyses root length and diameter of roots that have been washed free of soil, stained, and dispersed in shallow water in a clear tray. This software analyzes root morphology (length, area, volume, *etc*.), root topology, architecture, and color. A system comprised of the WinRhizo computer program and image acquisition components. Kuchenbuch and Ingram (2002) observed root growth of maize (*Zea mays* L.), which was grown in acrylic rhizotrons that held a 6-mm soil layer. The non-destructive measurement of total root length was performed by collecting images of the roots with a flatbed scanner and using RMS to analyze those images. To verify that roots observed at the surface of the rhizotron were representative of the total root system in the rhizotron, they were compared with destructive samples of roots that were carefully washed from the soil and analyzed for total root length and root diameter by using WinRhizo software. They found that there was a positive relation between visible and washed out roots.

WinRhizo displays the analysis over the image. The color used to draw the root skeleton indicates into which diameter class the part of the root has been classified. The same color is used for drawing the root distribution graphic above the image. The root distribution graphic displays the root length, area, volume or number of tips as a function of root diameter or color. Number and the width of root diameter classes are user-definable and can be changed at any time. Measurement data of the sample under analysis is summarized on screen and is available in detail in data files. The basic steps for root analyzing are following;

Root position: Simply place the roots directly on the scanner glass or in clear water-proof trays. Roots can overlap and do not need to be randomly distributed.

Acquire the image: WinRhizo controls the scanner or a digital camera directly. It is Twain compatible, meaning that it can get images from many scanners or cameras. It can analyze images stored in Tiff or Jpeg file formats.

Analyze the roots: The analysis is complete and roots found by WinRhizo are identified by colored lines in the image. The colors used for drawing the roots are coded according to their diameter. Root length and diameter are measured with an indirect statistical method (Tennant). Measurements are made continuously at each point along the root. Root overlaps, forks, and tips are taken into account to provide accurate measurements of length and area. Image edition is also available to override decisions made by the system.

Save the measurement data: WinRhizo automatically saves data following the analysis. Data files are in ASCII format, which can be read by most spreadsheets. Images can also be saved in files for later validations, analyses, or for visualization in other programs like word processors (www.regent.qc.ca/products/rhizo/Rhizo.html).

Root Measurement System (RMS)

Root Measurement System (RMS, Copyright, The University of Georgia) Version 2.5 was written in Visual Basic for MS Window 95 or higher to measure length and diameter of roots from digital images. Version 2.5 of RMS accepts images of any size in Jpeg or Tiff format. RMS recorded number of roots in an image and calculates total volume, total root surface area, and root length density (Ingram and Leers, 2001).

For minirhizotron images collected in a field study, an operator could analyze from 17 to 38 images hr^{-1} depending on number and length of roots in the images. With its speed, accuracy and versatility, RMS offers the possibility to analyze sufficient number of minirhizotron images to allow detection of treatment effects even under field conditions with large variability.

Quantitative Analysis of Color System (QuaCos)

QuaCos (Copyright, University of Georgia) is a program written in Visual Basic, analyzes red, green, and blue (RGB) values of pixels or groups of pixels in a digital images, producing a spreadsheet of values for the colors that user selects (Ingram *et al.*, 2001). Users may also choose the resolution of analysis from individual pixels to 500 × 500 pixel squares. QuaCos stores color values in a text file (ASCII), which may be read with either a word processor or a spreadsheet program. Color intensity was scored on a 0 to 255 scale. QuaCos operates under Windows 98 or higher operating systems and requires approximately 10 MB available on a hard drive.

Kuchenbuch and Ingram (2002) used the QuaCos program to quantify the changes of red-green-blue intensity of the soil color image pixels with related to soil water content. The analysis of 500 × 500 pixels for 25% of the image surface was

used to estimate average soil moisture, while the increment of 12 × 12 pixels over the entire image was used to estimate soil moisture with high spatial resolution. Only red value was inversely related with soil water content. As water content increased red values decreased.

Puntase (2005) also used the QuaCos program analyzing green color intensity of the minirhizotron digital images to estimate green fluorescent protein (GFP) *Aspergillus flavus* population density on root and pods of peanut. Full minirhizotron images were analyzed in 20 × 20 pixels, with the total number of 32 × 24 pixels groups for each image. The density of fungal population in the pod zone under water deficit was greater than under well watered conditions.

Carbon dioxide and Temperature

There is considerable concern about the increasing carbon dioxide concentration in the atmosphere, associated increases in temperature, and their effects on crop production. Since the beginning of the industrial revolution, the concentration of CO_2 has increased by 31%, by 150% in methane, and by 16% in nitrous oxide (Houghton *et al.*, 2001). At present rates of emission, CO_2 concentration is projected to be in the range of 540-970 µmol mol^{-1} by the year 2100, which will potentially increase global near-surface temperatures by 1.4 to 5.8^{o}C (Houghton *et al.*, 2001).

Effects of long-term CO_2 enrichment on physiological aspects of peanut yields

In general, elevated atmospheric CO_2 concentration increases plant biomass, root mass, and total leaf area (Rogers *et al.*, 1994), and increases leaf net

photosynthetic rates, decreases stomatal conductance, and increases leaf water-use efficiency (Gunderson and Wellschleger, 1994; Saxe *et al.*, 1998).

Bhagsari and Brown (1976) evaluated the effects of CO_2 on net photosynthesis and leaf characteristics of several peanut genotypes and found that net photosynthesis increased linearly for some genotypes as CO_2 concentration increased from 300 to 600 µmol mol^{-1}. Chen and Sung (1990) evaluated the effect of CO_2 at 340 (ambient) and 1000 µmol mol^{-1} from planting until seed filling in Virginia-type peanut. High CO_2 increased biomass and pod yields. Marketable seed yield was similar, however, because more than two-thirds of the pods on plants grown at 1000 µmol mol^{-1} CO_2 were unfilled. They hypothesized that increasing CO_2 during seed filling increased competition among developing seeds and pegs, thereby reducing seed growth. Stanciel *et al.* (2000) grew peanut in a hydroponic system at CO_2 concentrations of 400, 800, and 1200 µmol mol^{-1}. They found that plants grown at 800 µmol mol^{-1} CO_2 had net photosynthetic rates that were 29% greater than those of plants grown at 400 µmol mol^{-1}, whereas at 1200 µmol mol^{-1} CO_2 photosynthetic rates were 24% less than those of plants grown at 400 µmol mol^{-1}. Nevertheless, number of pods, pod weight, and seed dry weight per area increased with CO_2 enrichment from 400 to 1200 µmol mol^{-1}. The harvest index was 19% greater at 800 and 31% greater at 1200 µmol mol^{-1} compared to at 400 µmol mol^{-1}. In addition, as CO_2 concentration increased, stomatal conductance decreased, becoming 44% less at 800 µmol mol^{-1} and 50% less at 1200 µmol mol^{-1} than it was at 400 µmol mol^{-1}.

Effect of temperature on peanut growth

Temperatures in the tropics are near or above the optimum for production of most crops; therefore, crop yields are likely to decrease with even minimal increases

in temperature. The mean optimal air temperature range for vegetative growth of peanut is between 25 and 30°C, which is warmer than the optimum range for reproductive growth, which is between 22 and 24°C (Wood, 1968; Cox, 1979; Ong, 1984). Short or long-term exposures to air and soil temperatures above the optimum range can significantly reduce peanut yield (Golombek and Johansen, 1997; Prasad *et al.*, 1999a). Day temperature >34°C decreased fruit-set and resulted in fewer pods (Prasad *et al.*, 1999b). Decreased fruit-set at high temperatures resulted from poor pollen viability, reduced pollen production, and poor pollen tube growth, all of which led to poor fertilization of flowers (Prasad *et al.*, 1999b). Increasing daytime temperature from 26-30°C to 34-36°C significantly reduced numbers of subterranean pegs and pods and seed size, and reduced seed yield by 30-35% (Cox, 1979; Ong, 1984). Prasad *et al.* (2000) investigated the effects of daytime soil and air temperature of 28 and 38°C from the start of flowering until maturity and reported a 50% reduction in pod yield at high temperatures. Talwar *et al.* (1999) evaluated three peanut genotypes (ICG 1236, ICGS 44, and Chico) for their heat acclimation potential (HAP) and examined whether the growth, yield, and photosynthetic responses of these genotypes to temperature were related to HAP. They reported that all three genotypes maintained greater vegetative growth and photosynthetic rates when grown under the high temperature (35/30°C day/night temperature). However the high temperature regime adversely affected reproductive growth by increased flower abortion and decreased seed size. Craufurd *et al.* (1999) investigated the effect of high temperature and water deficit on water-use efficiency (WUE), carbon isotope discrimination (Δ), and specific leaf area (SLA). Five Spanish and three Virginia peanut lines were grown at mean temperatures of 27 and 34°C and at 50 and 100%

available soil water from first flowering until maturity. Virginia genotypes had greater total dry matter, water use, and WUE but were more sensitive to high temperatures than were Spanish genotypes. High temperature had no effect on total plant water use but decreased WUE and increased SLA.

Effect of temperature and CO_2 on plant growth and development

Within the non stress temperature range, plant development processes frequently respond to accumulated temperature such that phonological stages (e.g., leaf number, first flower, and first ripe fruit) are reached sooner at warmer temperatures (Johnson and Thornley, 1985). Most crop models used in climate change research incorporate temperature effects on development in some manner. This often leads to a prediction of reduced yields with increasing temperatures for determinate crop species, because plants reach maturity much sooner and the total growing season length is shortened. Wheeler *et al.* (1994) studied the effects of both temperature and CO_2 on development of carrot. They found that the increase in biomass at warm temperature was due almost entirely to temperature effects on rate of crop development, whereas increased biomass associated with elevated CO_2 was due partially to larger shoot and root size, as well as faster development. When plants were compared at the same leaf stage, there was almost no temperature effect, but still a 25% benefit in terms of biomass from elevated CO_2.

Both temperature and CO_2 also affect leaf morphology, root-to-shoot (R:S) ratio, and partitioning of C and N among plant organs (Wolfe *et al.*, 1998). In some cases increasing temperature and increasing CO_2 have similar effects, while in others the effects are in the opposite direction. An increase in CO_2 tends to have the opposite

effects on leaf development as an increase in temperature. Plants grown at warm compared with cool temperatures often have higher SLA (Wolfe, 1991; Wolfe and Kelly, 1992) and fewer cell layers (Boese and Huner, 1990). In contrast, elevated CO_2 has been reported to decrease SLA, lead 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).

The effects of temperature on whole plant growth response to elevated CO_2 also have been documented. Rawson (1995) observed that wheat grain yield increased only 7% with a CO_2 doubling in winter planting where temperature averaged about 12.5^{o}C. A temperature increases of 2^{o}C above ambient in these winter trials had little impact on CO_2 response. In contrast, in summer plantings when temperatures averaged about 20^{o}C, elevated CO_2 increased yields by 34%. Another study with wheat (Krezner and Moss, 1975) found no significant increase in wheat yield from CO_2 enrichment at the cool temperature regime of 13/17^{o}C (day/night). High temperature stress has a severe negative effect on reproductive development in some species, affecting growth and yield response to CO_2 enrichment. Gross and Kigel (1994) reported lowest pod-set when flower buds of bean were exposed to high temperature (32/37^{o}C) 6-12 days before and during anthesis. Prasad *et al.* (2002) reported that exposure of kidney bean to temperature >28/18^{o}C reduced photosynthesis, seed number, and seed yield at both ambient (350 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) CO_2 levels. Elevated CO_2 did not affect seed size but temperature >31/21^{o}C linearly reduced seed size by 0.07 g ^{o}C^{-1}. Prasad *et al.* (2003) also found that at ambient CO_2, seed yield of peanut decreased by 14-90% as temperature increased from 32/22^{o}C to 44/34^{o}C. Similar decreases in seed yield

occurred at temperature above 32/22^{o}C with elevated CO_2 despite greater photosynthesis and vegetative growth. Baker and Allen (1992) reported that grain yield of indica-type rice cultivar IR-30 declined by about 10% per each 1^{o}C increase in average day-night air temperature beyond 26^{o}C and reached zero yields at 37^{o}C.

Response of peanut plant to drought

Water availability is limited in many parts of the world. Drought restricts plant growth and crop production more than any other single environment factor. Drought affects nearly every aspect of plant growth and most physiological processes.

Relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration, leaf temperature, and canopy temperature are important parameters involved with water relations in peanut. Relative water content of leaves is higher in initial stages of leaf development and declines as the dry matter accumulates and leaf matures (Jain *et al.*, 1997). Obviously, stressed plants have lower RWC than non-stressed plants. Non-stressed plants had RWC range from 85-90%, while droughted plants had RWC as low as 30% (Baru and Rao, 1983). Day-time leaf and canopy temperatures of irrigated plants are generally less than ambient air temperature but rainfed plants often have a higher canopy temperature than ambient air temperature, which indicates water deficit in rainfed plants (Erickson and Ketring, 1985). Transpiration rate generally correlates with incident solar radiation when sufficient water is available to meet plant needs. However, droughted plants transpire less than unstressed plants. Subramaniam and Maheswari (1990) found that leaf water potential, transpiration rate, and photosynthetic rate decreased progressively with increasing

duration of water stress, while stomatal conductance decreased steadily during the stress period.

Drought also reduced crop photosynthesis due to reduced stomatal conductance and reductions in leaf area. As moisture deficit increases, both transpiration loss of water and uptake of carbon dioxide are reduced. Water deficit also decreases the conductance of mesophyll cells, which further slows uptake of carbon dioxide and reduces photosynthesis. Bhagsari *et al.* (1976) observed large reductions in photosynthesis and stomatal conductance as the relative water content of peanut leaves decreased from 80 to 75%. The main effect of a soil water deficit on leaf carbon exchange rate is exerted through stomatal closure. They reported that withholding water in potted plants for 3 days can reduce carbon exchange rate, decrease transpiration, and decrease stomatal conductance. Allen *et al.* (1976) found reduced stomatal resistance by 7 days after stress and significant differences within 10 days between stressed and non-stressed plants. The long-term effect of soil water deficit on canopy CO_2 assimilation is a reduction in leaf area. Readdy and Rao (1968) reported that severe drought decreased the levels of chlorophyll a, b, and total chlorophyll. However, mild drought increased chlorophyll content (Moreshat *et al.*, 1996).

Almost all plant metabolic processes are affected by water deficit. Metabolic changes in response to water deficit include reduction in protein synthesis, accumulation of organic acids such as malate, citrate, and lactate accompanied by accumulation of proline and sugar. Severe water deficits cause decreases in enzymatic activity. Complex carbohydrates and proteins are broken down by enzymes into simpler sugars and amino acids (Pandey *et al.*, 1984). Accumulation of soluble

compounds in cells increases osmotic potential and reduces water loss from cells. Reddi and Reddy (1995) observed that accumulation of proline is greater in the later stages of drought and therefore its concentration is considered a good indicator of moisture deficit. Moreover, exposure of plants to low water potential often leads to loss of cell turgor and plants undergo osmotic adjustments by the rapid accumulation of abscisic acid.

Water deficits reduce the number of leaves per plant and individual leaf size. Leaf longevity and leaf area duration are reduced by decreasing soil water potential. Leaf area expansion depends on leaf tugor, temperature, and assimilates available for growth, all of which are affected by drought. Leaf and stem morphology are altered by water deficit. Continuous water deficit results in fewer and smaller leaves, which have smaller and more compact cells and greater specific leaf weight (Chung *et al.*, 1997). Main axis and cotyledonary branches are shorter for peanut plants under water deficit than for non-stressed plants. Soil water deficit reduces internode length more drastically than node number. Bell *et al.* (1993) studied the factors influencing dry matter partitioning in four diverse peanut cultivars. Rates of dry matter accumulation in pods varied significantly with both cultivar and sowing date. Within cultivars, much of this variation could be attributed to variation in crop growth rate (CGR) during the critical pod addition period. The proportion of current assimilates distribution to pods depended on inherent cultivar characteristics, and also correlated well with current CGR relative to the CGR during pod addition. Relative amounts of assimilate distributed between vegetative and reproductive parts were not influenced by plant density or spatial arrangement of plants. All cultivars appeared capable of

remobilizing stored assimilate to maintain near constant rates of dry matter accumulation in pods (Pandey *et al.*, 1984).

Roots are critical for plant survival in dry environments. Because of their direct contact with drying soil, roots may mediate drought resistance through various major physiological processes. In peanut, roots grow rapidly during germination and seedling stage and within 5 or 6 days after sowing, the taproot may grow 10-16 cm deep and develop a number of lateral roots. Ketring and Reid (1993) found that root length density significantly increased at 10 cm depth until 80 days. At 45-50 days, root had penetrated to a depth of 120 cm and spread laterally at least 46 cm. Gregory and Reddy (1982) found that the total root length of cultivar Robout 33-1 followed a sigmoid growth curve and peaked at 68 days after sowing. Root growth of peanut is influenced by soil moisture. Water deficit stimulates the growth of roots into deeper soil layers (Narasimham *et al.*, 1977). Pateña (2000) observed that under water deficit, peanut plants that took up water from deeper soil layers could continue growth processes longer than plants with shallow root systems. Drought resistance in crop plants was associated with deep root systems, larger root density, and greater extraction of water from deeper layers of the soil profile (Senthong and Pandey, 1998; Boonpradub, 2000). While deep roots enable plants to resist water deficit, the root system must be established before stress to provide this resistance because water deficit restrict root growth (O'Toole and Bland, 1987). Allen *et al.* (1976) concluded from measured soil water extraction, that during water stress roots in deeper soil layers continued to grow deeper even though vegetative growth appeared to stop. They further stated that peanut roots effectively extracted soil water from a depth of at least 180 cm in fine soil. Simmonds and Ong (1987) found that the cultivar Robut

33-1 more rapidly extracted water from deeper layers when grown at high vapor pressure deficits than when grown in humid air. Devries *et al.* (1989) reported that cultivar Florunner had greater root length density in deeper layers (60-150 cm) during drought. Florunner exhibited greater capacity for deep rooting at 55 days after sowing than that of soybean or cowpea, especially when grown under drought. All these traits contribute to peanut's ability to avoid the effects of drought. Pandey *et al.* (1984) showed that peanut had greater root length density deeper in the soil than other legumes when grown under drought. Sabale and Khuse (1989) observed the highest root lengths when available soil moisture from 80-85% field capacity. They also reported that spraying antitranspirants did not influence either root length or root volume. Meisner (1991) used two non-destructive methods, a rhizotron and minrhizotron to observe peanut root growth under 30-day drought periods beginning 20, 50, 80, and 110 days after sowing. Root growth was reduced significantly by drought during 20-50 days after sowing compared with irrigated control in the rhizotron study, however, such differences were not observed in plants grown in minirhizotrons (Meisner and Karnok, 1991). Meisner and Karnok (1992) observed root growth on rhizotron glass every week and found that peanut root system, regardless of water stress, did not exhibit signs of senescence. Root color and florescence of the root system did not change throughout the season at all depths indicating viable root system during water deficit may contribute to the crop's drought resistance (Sanders *et al.*, 1993). Greater partitioning of assimilates to the root system before pod set and a root system that maintains itself for a long period should confer advantages over plants whose roots are continually dying and regrowing during reproductive development.

Drought also influences yield attributes and yield of peanut. The level of yield reduction by a water deficit depended on the degree, duration, and timing of water deficit (Begg and Turner, 1976). The start of flowering was not delayed by drought (Boote and Ketring, 1990). The rate of flower production was reduced by drought during flowering but the total number of flowers per plant was not affected because there was an increase in the duration of flowering (Mirsner and Karnok, 1992). A significant burst in flowering on alleviation of stress was a common feature in the pattern of flowering under moisture deficit, particularly when drought was imposed just prior to reproductive development (Janamatti *et al.*, 1986). When stress was imposed during 30-45 days after sowing the first flush of flowers produced up to 45 days did not form pegs, however, flowers produced after re-watering compensated for this loss (Gowda and Hegde, 1986). Peg elongation was reduced due to drought (Boote and Ketring, 1990). Pegs failed to penetrate effectively into air-dry soil, especially in crusted soils. Skelton and Shear (1971) reported that adequate root zone moisture could keep pegs alive until pegging zone moisture content was sufficient to allow penetration and initiation of pod development. Adequate pod zone moisture was critical for development of pegs into pods and adequate soil water in the root zone could not compensate for lack of pod zone water for the first 30 days of peg development. Sexton *et al.* (1997) reported that peanut fruit growth was sensitive to surface soil (0-5 cm) conditions because of its subterranean fruiting habit. A dry pegging zone soil delayed pod and seed development. Soil water deficit in the pegging and root zone decreased pod and seed growth rates by approximately 30% and decreased weight per seed from 563 to 428 mg. Peg initiation growth during drought demonstrated an ability to suspend development during soil water deficit and

to re-initiate pod development after the drought was relieved (Sexton *et al.*, 1997). Pod and seed development are progressively inhibited by drought as a result of insufficient plant tugor and lack of assimilates. These developmental stages can also be delayed by lack of soil water in the pod zone (Boote and Ketering, 1990; Stirling and Black, 1991). Pod dry weights were significantly reduced by a 30-day water deficit during pod development stage (Meisner and Karnok, 1992). Drought reduced pod yield primarily by decreasing the duration of the pod development phase (Stirling and Black, 1991). Prabawo *et al.* (1990) reported that irrigation applied before and after early pod filling stages increased pod yields of Spanish type peanut to 2.4 t ha^{-1} compared with 0.53 t ha^{-1}in a dry land crop. Suther and Patel (1992) found that pod yield was higher with 80% available soil water than with 20% available water. No pods were formed when plants grown in water-saturated soil (Bailey and Biosvert, 1991). Stirling and Black (1991) concluded that the major cause of variability in pod yield and harvest index in semi-arid tropics was the delay between peg initiation and onset of rapid pod growth. The reason for this is that once pods were initiated, the proportion of dry matter allocated to reproductive sinks was relatively constant.

Aspergillus flavus associated with aflatoxin production

Aspergillus flavus is a filamentous fungus that is known to occur mostly in soil, but it is also found in plant products, particularly oil-rich seeds and in living plants. This fungus is notorious for producing aflatoxin, which is toxic to humans and animals (CAST, 1976).

Colonies of *A. flavus* are yellow to green on Czapek's agar medium. The hyphae are well developed, profusely branched, septate, and hyaline. The conidial head is globose to radiate or columnar, very light yellow-green, deep yellow green, olive-brown, or brown. Conidiophores are colorless, usually roughened but vary form smooth or nearly so to coarsely roughened. Vesicles are globose or subglobose at maturity in species with large heads, remain clavate shaped in species with small heads, and are fertile over most of their surface. Sterigma is uniseriate or biseriate with both conditions commonly seen in the same strain or on a single vesicle. Conidia in most species are globose or subglobose when mature with roughening conspicuous or almost absent and often showing considerable intra strain variability in size. Sclerotia are dark red brown to purple brown or black at maturity, globose, subglobose or vertically elongate (Thom and Raper, 1945; Raper and Fennel, 1973).

The life cycle of *A. flavus* starts from spore germination and ends with spore formation in conidial structures. The duration of the life cycle depends largely on the kind of substrate present and conditions in the growing environment. Under field condition, nutrients and immediate microclimate are not ideal, as in the laboratory, and spores will not germinate under unfavorable conditions. Spores are able to withstand a period of unfavorable conditions which would be fatal to the vegetative

parts of the fungus. Running water is important for short distance transport of spores. Convection air currents are responsible for local spread of dry spores. Insects, animals, and equipment provide other avenues for conidial spore dispersal.

A spore germinates when it falls on a suitable moist substrate with other conditions favorable. The general requirements for germination of spores are: suitable temperature, adequate moisture supply, adequate oxygen supply, suitable pH, and viable spores. Only a small portion of fungal spores reaches a favorable substrate with favorable conditions for germination (Hawker, 1950). During germination, a spore forms a bulge, which then elongates to form a slender thread or germ tube. The germinated spore becomes vegetative and forms a thin but close textured basal mycelium.

Abundant conidial structures, forming conidiophores are then produced directly from the substrate mycelium. Conidiophores are heavy walled and coarsely roughened and are usually less than 1 mm in length have stalk diameters immediately below the vesicles raging from 10 to 20 µm. Conidiophores are upright, simple, terminating in a globose or clavate swelling, with the phialides at the apex radiating from the entire surface (Barnett, 1960). Young conidial heads have yellow shades near strontium yellow or yellowish citrine. As the conidial heads age, their color changes to dark yellow shades and finally become deep grape green. Conidial heads radiate, splitting into several poorly defined columns less than 600 µm in diameter. Spores are born in the conidial head. The spores ripen and mature, ready to start the next cycle (Raper and Fennel, 1973).

Under favorable conditions of high temperature and humidity, *A. flavus* grow on certain foods and feeds, often producing aflatoxins. Water deficit and high temperature stimulate *A. flavus* infection of developing peanut pods. Infection and heavy aflatoxin contamination are favored at 25 to 31^{o}C with 0.85 to 0.95 of water activity (a_w) in pod during drought (Molina and Giannuzzi, 2002; Sautour *et al.*, 2002). Drought could decrease metabolic activity in the seeds, high temperature could affect the development of microbial competitors in the geocarposphere, and both could affect phytoalexin production (Mehan *et al.*, 1991). Other environmental factors that influence growth of *A. flavus* and production of aflatoxin are linoleic acid, light, and pH (Calvo *et al*., 1999; Molina and Giannuzzi, 2002).

Sources of *A. flavus* spores that invade peanut may contaminate from the air, soil surface, or within the soil. All developing peanut plants are contaminated with this fungus but the potential for peanut pod invasion increases when a peg is infected before pods form. Peanut peg infection could start from flowers and developing pegs before the peg penetrates into the soil. Airborne spores may fall on flowers or developing pegs and germinate with favorable conditions. Yingthongchai (1994) reported that both flowering and pod maturing stages were the most critical growth stage for *A. flavus* infection in peanut, but the degree of infection varied among resistant cultivars. Manzo and Misari (1989) and Pitt (1989) reported that *A. flavus* can infect peanut plants during the flowering and pod filling stages.

The possible avenue of infection is through the developing peg when the peg contacts and penetrates the soil surface. *A. flavus* spores at the soil surface may invade elongating pegs as they penetrate the soil. Because the soil has a rich substrate media that can support *A. flavus* growth, and possibly more favorable moisture conditions

than those on floral surfaces, conditions may be more favorable for spore germination at the soil surface than on flowers. However, competing microflora can retard or inhibit *A. flavus* infection. *A. flavus* inoculum available in the rhizosphere within and around the pegging depth is the next source of infection as they can penetrate through the soil with *A. flavus* adhering through the peg surface. Griffin (1972) showed that two weeks after pegs entered soil inoculated with *A. flavus*, dilution plate analysis from the 0.5-mm soil layer surrounding the peg produced a mean fungus population more than four times greater than the population from comparable soils located away from developing pegs. This observation is probably because the nutritional stimulus for *A. flavus* germination in the developing peg surface was not sufficient. Thus, the potential for infection can only be attributed to the very close proximately of the inoculum when the pod is formed.

The next possible pathway for *A. flavus* infection of peanut pod is through mechanical damage or breaks along the pod surface, which expose inner pod layers. Griffin (1972) mechanically injured 4 to 6 mm^2 of pod surface area treated the injured areas with a 100 count conidia. Results showed 56 to 63% conidial germination on the pod surface. There was no germination on plain soil with similar conidial treatment. The high germination rate was observed on pod surfaces for a temperature range from 30 to 35^{o}C. There was low *A. flavus* spore germination at 20^{o}C. These results of Griffin (1972) show that substrates found at the surface of injured pods can induce germination and that *A. flavus* may require an opening to penetrate the pod surface layer. After the pod surface layer is invaded through cracks, seed infection follows. Schroeder and Ashworth (1965) reported that aflatoxin in Spanish peanut seeds were only detected in seed from broken pods. This observation suggests that seed invasion

only occurs when openings are present from the protecting pods. The aflatoxin concentration is greater on seeds in pods with growth cracks than seeds in pods with mechanical injury. Growth cracks occur normally in pods. Thus, even when other mitigating pod injuries are absent, pod openings for *A. flavus* infection develop.

A comparison of pod and seed screening methods showed seed screening methods were equally or more effective than the pod screening methods in identifying genotypes resistant to *Aspergillus* spp. The greatest concentration of *A. flavus* invasion is along the surface of the seed coat of the seed. Suriyong (1997) reported that seed coat thickness of peanut has no correlation with the resistance to *A. flavus*. Resistant genotypes have an unchanged seed coat structure. In contrast, susceptible genotypes show a breakdown in seed coat structure when infected with *A. flavus*. This phenomenon indicated that the seed coat of resistant genotypes might contain some chemical or tissues that block the pathway and inhibit the spread of the fungus.

Green fluorescent protein (GFP) *Aspergillus flavus*

A gene for a natural fluorescent protein from a jellyfish (*Aequorea victoria*) was tested for its ability to differentiate genetically-modified *A. flavus* from wild type fungus. This gene for green fluorescent protein (GFP) allowed detection of genetically-modified fungus. Detection of infection with the modified *A. flavus* could be performed quickly and easily without expensive equipment. No microscope or light filters were required. Any ultra-violet light source was sufficient for detection of the GFP gene (www.nal.usda.gov). When illuminated with ultra-violet (UV) light (350 to 380 nm), the GFP fluoresces green. Wangeli *et al.* (1999) suggested that GFP

containing transformants could be useful in screening for the resistance of corn genotypes to aflatoxin accumulation and making screening faster. Ingram *et al.* (1999) observed that GFP *A. flavus* populations, as estimated by amount of fluorescence, increased at peanut root and pod surfaces and that particularly under dry conditions. The population of *A. flavus* appeared to be greater on roots and pods of drought susceptible than drought resistant peanut genotypes. Puntase *et al.* (2006) reported that the maximum infection with GFP *A. flavus* was achieved by spraying an aqueous spore suspension over shoots and flowers of peanut. They also found fluorescing network hyphae on ovules inside the peanut pegs as observed with a UV-illuminated microscope.

Drought and aflatoxin contamination in peanut

In many parts of the world, peanut is grown under rainfed conditions. The crop often suffers from drought of varying intensity and duration during the growing season (Dwivedi *et al.*, 1996). Adverse effects of drought on pod yield and quality of peanut are well documented (Stansell *et al.*, 1976; Nageswara Rao *et al.*, 1985; Nageswara Rao *et al.*, 1989). Several researchers have found that peanut sensitivity to water deficit depends on the stage of growth (Martin and Cox, 1977; Pallas *et al.*, 1979). Early and late season droughts are not as detrimental to yield as drought during pegging and pod set. However, drought during pod maturity poses the greatest risk of aflatoxin contamination. Thus, drought adversely affects both yield and crop quality depending on the growth stage at which it occurs. Several studies have been conducted to understand the predisposition of peanut to aflatoxin contamination after

drought and on screening peanut germplasm to identify genetic sources that are resistant to invasion by aflatoxin producing *Aspergillus* (Cole *et al.*, 1985; Sander *et al.*, 1993).

Drought, especially during the later part of the growing season (Holbrook *et al.*, 1944a), and temperatures from 25 to 38^{o}C (Jackson, 1965) have been associated with aflatoxin contamination of peanut. Wilson and Stansell (1983) conducted studies on the relationship of water deficit intensity and timing with pre-harvest aflatoxin contamination of peanut. In 2 of 4 years they found significantly more aflatoxin in peanut seeds when a terminal drought was imposed at least 40 days preceding harvest. Sander *et al.* (1985) also found that the threshold duration for terminal drought causing preharvest aflatoxin contamination of peanut by *A. flavus* was more than 20 days and possibly less than 30 days before harvest. Cole *et al.* (1985) and Sanders *et al.* (1985) reported that high *A. flavus* invasion may be found without the presence of aflatoxin, suggesting that invasion and subsequent growth and aflatoxin contamination are separate processes or at least regulated in different ways.

Although drought predisposes peanut to aflatoxin contamination, the mechanism of drought induced aflatoxin production is not known. Southern Runner peanut cultivar was found to be partly resistant to aflatoxin contamination when subjected to late season drought, a trait that may be correlated with drought tolerance (Cole *et al.*, 1993). This decrease resulted from reduced metabolic activity due to a decline in pod water content, which increases susceptibility of peanut to fungal invasion. Invasion of *Aspergillus* species occurs primarily during drought and is associated with elevated soil temperature (Dorner *et al.*, 1989). Pre-harvest *A. flavus* invasion and aflatoxin contamination are greater in small, immature seeds than in

mature seeds. This information led to the suggestion that the seed resistance mechanisms that may prevent *A. flavus* growth and aflatoxin production fail first in immature seeds in response to drought and heat stress (Cole *et al.*, 1985). Although water deficit predisposes peanut to *Aspergillus* species invasion, aflatoxin contamination depends upon maturity status, condition of the seed, and duration of stress. This result would suggest that, susceptibility of peanut to aflatoxin contamination is inconsistent and varies depending upon genotype, environment, maturity, and severity of stress.

Aflatoxin resistance in peanut may result from resistance to *Aspergillus* spp. infection or resistance to aflatoxin production. Partial resistance to aflatoxin has been found in a few peanut genotypes and methods have been developed to screen field-grown peanut germplasm for aflatoxin resistance (Will *et al.*, 1994; Anderson *et al.*, 1995; Clavel, 1998). It is difficult to screen germplasm directly for aflatoxin resistance because *Aspergillus* spp. infection occurs mostly in plants that have been weakened by stress, particularly drought, and because even susceptible genotypes have relatively few pods that are infected and contaminated under stress. Thus, rather than attempting to screen germplasm directly for aflatoxin resistance, it may be possible and for more efficient to develop aflatoxin resistant germplasm indirectly, that is through screening for drought resistance or other traits that confer aflatoxin resistance (Holbrook *et al.*, 1994b).

Under water deficit, peanut plants that are able to take up water from deeper soil layers can continue growth processes longer than plants with shallow root systems (Pateña, 2000). Drought resistance in crop plant has been associated with deep root systems, larger root density, and greater extraction of water from deeper soil

layers (Boonpradub, 2000; Senthong and Pandey, 1998). While deep roots enable plants to resist water deficit, the root system must be established before stress to provide this resistance because water deficit restricts root growth (O'Toole and Bland, 1987). Thus, screening for deep roots by observation with minirhizotron (Pateña and Ingram, 2000) may advance efforts in developing aflatoxin resistant germplasm as well as the direct benefits from more stable yields of drought resistant varieties under rainfed condition. Recent research (Ingram *et al.*, 1999; Pateña, 2000) showed that a minirhizotron system also could be used to observe *A. flavus* growth on peanut roots and pods *in situ* with peanut plants grown in containers were inoculated with a strain of GFP *A. flavus*. Ingram *et al.* (1999) observed that *A. flavus* populations, as estimated by amount of fluorescence, increased at peanut root and pod surfaces and that particularly under dry soil conditions, *A. flavus* appeared to be greater on roots and pods of drought susceptible peanut genotypes than on roots and pods of drought resistant genotypes.

Seed biochemical components in relation to *Aspergillus flavus* growth and aflatoxin production

Aflatoxins are known carcinogenic compounds and regulations limiting their concentrations in food and feed are present in at least 15 countries worldwide (Haumann, 1995). The aflatoxigenic fungi *Aspergillus flavus* Link ex. Fries and *A. parasiticus* Speare infect a wide variety of crops, all of which produce oil-rich seeds. Aflatoxin production is affected by many biotic and abiotic environmental factors such as temperature, water status, pH, nutritional conditions (Payne and Brown, 1998; Chang *et al.,* 2000), and interactions between host and invading fungi. Carbon and nitrogen sources play a vital role in the regulation of aflatoxin production (Dutton, 1988; Payne and Brown, 1998; Chang *et al.*, 2000; Aziz *et al.*, 2002).

When simple sugars such as glucose, sucrose, maltose, and galactose provide the growth substrate, *A. flavus* produces more aflatoxin than when complex carbohydrates, such as starch and peptone, provide the growth substrate (Adyne and Mateles, 1964; Payne and Brown, 1998). Glucose and sucrose have similar effects on inducing aflatoxin formation. Although the metabolic basis for this phenomenon is not known, available evidence suggests that glycolysis has an important role in aflatoxin biosynthesis and that fermentable sugars are optimal carbon sources for aflatoxin production (Abdollahi and Buchanan, 1981a, 1981b). Trace elements also are essential for the growth of *A. flavus* and for aflatoxin production. When *A. flavus* is grown in Zn-deficient medium, aflatoxin production is low, conversely the presence of Mn in the medium inhibits aflatoxin production (Luchese and Harrigan, 1993). Failla *et al.* (1986) showed a positive correlation between aflatoxin production and the

Zn content in maize kernels, but they found no correlation between Mn content and aflatoxin production. Pitt *et al.* (1991) found that a higher Ca content in the seed coat of peanut decreases the growth of *A. flavus* during field-drying. Conversely, when seeds are oven-dried there is an increase in fungal growth with increased seed coat Ca levels. Fernandez *et al.* (1997) also found that the development of *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. was decreased and even suppressed when the Ca content of the seed coat was increased from 2.2 to 5.5 g kg^{-1}.

The effects of lipids on fungal growth and aflatoxin production have been studied in *A. flavus* and *A. parasiticus* (Faneli *et al.* 1983, 1995; Fanelli and Fabbri, 1989; and De Luca *et al.* 1995). Lipids in seeds can enhance the growth of *A. flavus*, but the stimulation of aflatoxin by seed lipids correlates with potential for lipoperoxide formation (Febbri *et al.*, 1983; Luchese and Harrigan, 1993; Reddy *et al.*, 1993; Burrow *et al.*, 1997). Lipoperoxides are produced in many host-pathogen interactions by the oxidation of unsaturated lipids. Seeds with high levels of polyunsaturated lipids have a higher potential for lipoperoxidation than do seeds with monounsaturated lipids. In support of this hypothesis, Fabbri *et al.* (1983) showed that the addition of free radical generators to the culture medium increased aflatoxin production by *A. flavus*, suggesting that aflatoxin protects the fungus against the free radicals associated with lipoperoxides. Saturated free fatty acids support fungal growth and aflatoxin production, while unsaturated free fatty acids inhibit fungal growth (Fanelli and Fabbri, 1989). Lypoperoxidation of unsaturated free fatty acids produces lipoperoxides, which are presumed to inhibit fungal growth and reduce aflatoxin biosynthesis (Fanelli and Fabbri, 1989). Ergosterol oxidation also induces both fungal growth and aflatoxin production (De Luca *et al.*, 1995). Doehlert *et al.*

(1993) found that if soybean homogenates were supplemented with large amounts of lipase, linoleic acid, or linolenic acid, volatiles that inhibit fungal spore germination were generated. Oleic acid had no effect compared with untreated homogenates. Hexanal, a product of lipoxygenase pathway with known antifungal activity was the major volatile generated from lipase-treated homogenates. Burrow *et al.* (1997) observed that 13*S*-hydroperoxy linoleic acid significantly decreased aflatoxin production when introduced into growth media at 24-h intervals. Calvo *et al.* (1999) showed that development of asexual spores, cleistothecia, and sclerotia formation of *A. nidulans*, *A. flavus*, and *A. parasiticus* were affected by linoleic acid and light. They also suggested that light and linoleic acid may be significant environmental signals for fungal development.

Nitrogen source can also influence the aflatoxin level produced in culture by *A. flavus* (Luchese and Harrigan, 1993). Complex organic N sources such as peptone and yeast extract result in higher aflatoxin production. Though relatively simple N compounds, proline and asparagine also stimulate aflatoxin production. In contrast, high nitrate concentrations suppress aflatoxin production.

Proteins from kernel extracts of maize (*Zea may*) genotypes were analyzed by polyacrylamide gel electrophoresis. Consistent differences in protein profiles were detected among genotypes. Several proteins were present in greater concentration in resistant genotypes, whereas others were present only in susceptible genotypes. Extracts of maize kernel aflatoxin resistant genotypes showed markedly greater antifungal activity against *A. flavus* than did kernel extracts from susceptible genotypes (Guo *et al.*, 1998). Huang *et al.* (1997) identified two proteins that may contribute to resistance to aflatoxin production from resistant maize inbred Tex6. One

protein with a molecular mass of 28 kDa inhibits *A. flavus* growth, while a second protein with a molecular mass >100 kDa inhibits toxin formation with little effect on fungal growth. Maize genotypes ether resistant or susceptible to *A. flavus* were extracted for protein analysis using a pH 2.8 buffer. A 14-kDa protein was presented in relatively high concentrations in 7 resistant genotypes, but was present only in low concentrations in 6 susceptible genotypes. The N-terminal sequence of this 14-kDa protein showed 100% homology to a maize trypsin inhibitor. The 14-kDa protein purified from resistant genotypes also demonstrated *in vitro* inhibition of trypsin activity and the growth of *A. flavus*. This protein is the first demonstration of antifungal activity in maize. Chen *et al.* (1998) suggested that the expression of this protein may be related to the genotype differences in resistances to *A. flavus* infection and subsequent aflatoxin contamination. Maureen *et al.* (2000) found three maize-derived volatile compounds, *n*-decyl aldehyde, hexanol, and octanol, reduced radial growth of *A. parasiticus*, but only *n*-decyl aldehyde significantly inhibited aflatoxin biosynthesis. However the maize protein extracts may contain both elicitors and inhibitors of aflatoxin B1 production (Costa *et al.*, 1988). The antifungal activity of base-soluble proteins (BSP) and methanol-soluble polysaccharides (PS) from *A. flavus* resistant and susceptible maize were investigated in *in vitro* studies. Bioassay of fungal growth inhibition in agar media showed antifungal activity by proteins and polysaccharides only from the susceptible genotype (Neucere and Godshall, 1991).

Because plants do not have an immune system they must rely on other mechanisms to protect themselves from fungal infection. These mechanisms include synthesis of inhibitory compounds such as phenols, melanins, tannins, and phytoalexins that can directly inhibit fungal growth (Huynh *et al.*, 1992). Zambettakis

(1983) showed that seeds harvested from different peanut cultivars vary widely in their resistance to *A. flavus* when inoculated with a spore suspension and that this variation correlated with resistance to the fungus in the field. After that they confirmed variation in resistance among peanut cultivars they showed that resistance was correlated with a potential for rapid accumulation of stilbene phytoalexins on wounding. Wotton *et al.* (1987) showed that phytoalexin accumulation in response to wounding was decreased if the plant subjected to drought. Mohanty (1991) found phytoalexins produced by 20 peanut genotypes following mechanical damage of seeds varied in amount and composition. Some genotypes produced one major phytoalexin component, while the other genotypes produced as many as 7 major components. High phytoalexin producing genotypes used more methionine-rich protein (present in seed) than other genotypes, and it is suggested that this protein or its breakdown products may play a role in phytoalexin production. Azaizeh and Pettit (1987) reported that level of tannin compounds in peanut seed coats and cotyledons differed among genotypes, with levels being higher in seed coats. Some compounds significantly decreased the growth of *A. parasiticus* and inhibited the production of aflatoxin. There was no overall correlation between the peanut genotypes and the influence of tannin extracts on *A. parasiticus* growth and aflatoxin production. However, correlation was higher for specific genotypes (Azaizeh *et al.*, 1990). Both the growth of *A. parasiticus* (NRRL2999) on Potato dextrose agar (PDA) and production of aflatoxin in liquid culture were inhibited by the tannins (Lansden, 1982).