GENERAL INTRODUCTION

Preharvest and postharvest infection of peanut (*Arachis hypogaea* L.) by *Aspergillus flavus* is one of the more serious problems in Thailand and throughout the world. This fungus causes extensive economic losses either by destroying the plant or by contaminating peanut seeds with carcinogenic aflatoxins which can cause serious health hazards to human and domestic animals. After aerial fertilization of the peanut flower and extension of the peg into the soil, the peanut fruit develops in a subterranean environment and may be invaded by many species of soil microorganisms during its subsequent growth and development. Environmental conditions and management practices during production, harvest, handling, and storage may affect nature and degree of fungal contamination.

In many parts of the world, peanut is grown under rainfed conditions. The crop often suffers from drought of varying intensity, timing, and duration. Drought is a complex combination of stresses because of both water deficit and high temperature. Heat stress may occur either when air temperature is above the optimum, about 30^{o}C for development processes or 25^{o}C for growth processes in peanut (Williams and Boote, 1996). Drought may occur during any stage of peanut development and increases crop predisposition to infection by *A. flavus* and subsequent aflatoxin contamination, due to negative effects on host physiology. Yingthongchai (1994) reported that drought during flowering and pod maturing developmental stages of peanut led to the greatest susceptibility to *A. flavus* infection. Drought has been found to increase the number of *Aspergillus* spores in the air. Therefore, when drought occurs during pollination, the increasing inoculum load (spore in the air) may increase

the chances of flower infection. Preharvest invasion by *A. flavus*, as well as extensive preharvest formation of aflatoxins, can occur in visibly undamaged peanut pods. Although colonization of peanut pods by *A. flavus* can occur either before or after harvest, and this fungus can infect pods in the soil during some stage of fruit development or earlier during the flower or aerial peg formation, little is known of the nature and mechanisms by which this fungus infects peanut flowers.

Fungal growth and mycotoxin biosynthesis are affected by several factors, including substrate composition, moisture, temperature, pH, and stresses such as drought and associated growth of other molds or microbes.

At the present, the rise in atmospheric CO_2, due mainly to fossil fuel combustion and land use change, is an undisputed fact. This ongoing CO_2 increase has important implications for vegetation. Carbon dioxide is substrate for photosynthesis and, when elevated, both carbon assimilation and water use efficiency generally increase. Stimulation of root system development associated with increased growth implies more rooting, which, in turn, implies the possibility of increased water and nutrient capture. Microbes mediate carbon and nutrient flows within the soil, and CO_2-induced changes in the structure and function of plant root systems may lead to changes in the microbiology of both rhizosphere and soil. Enhanced plant growth further suggests greater delivery of carbon to soil, and thus potentially greater soil carbon storage. Carbon dioxide-induced changes in plants will affect the structure and function of rhizosphere and soil microorganisms. As pathogens, symbionts, and decomposers, microbes exert a strong influence on carbon and nutrient cycling in plant/soil systems. Change in plant structure (Prior *et al.*, 1995), physiology (Roger *et al.*, 1994), and phytochemistry (Pritchard *et al.*, 1997) brought about by elevated

atmospheric CO_2 may alter plant-microbe interaction in the soil. In addition, root exudation, which can increase in plants grown under CO_2 enrichment (Norby *et al.*, 1987), might provide substrate for *A. flavus* growth in the soil, which may increase peanut pod infection by this fungus.

This study proposes to determine the combined effects of elevated temperature and CO_2 on peanut growth and *A. flavus* infection. Studies will include monitoring differential plant response to water deficit and *A. flavus* infestation by drought-tolerant and drought-susceptible peanut genotypes and also examine the pathway of *A. flavus* infection into the peanut flowers, ovary, and aerial pegs which relate to preharvest infection.

The objectives of this research were to: i) observe the growth and development responses of shoots and roots of peanut under different combinations of atmospheric CO_2 concentration and temperature, ii) determine the effect of different CO_2 concentration and temperature on *A. flavus* infection, iii) evaluate effects of different levels of water deficit on shoots and roots growth of peanut genotypes, iv) determine the responses of peanut genotypes to *A. flavus* infection under different levels of water deficit, v) determine the biochemical responses of pods of peanut genotypes to *A. flavus* infection under drought stress condition, and vi) observe the pathway of green fluorescent protein (GFP) *A. flavus* invasion through peanut flower using fluorescence under UV-illuminated microscope.

This research consists of three experiments. Under future climate change scenarios, it is most likely that plants will be exposed to a combination of both higher temperature and CO_2 concentration (Rosenzweig and Hillel, 1998). The first experiment deals with the growth and development of peanut shoots and roots of and

also *A. flavus* infection in response to the combination of two levels of air temperature and three levels of CO_2 concentration. This research work comprised a long-term experiment, in which plants were grown in 20-L containers for a total period of 112 days and a short-term experiment, in which growing plants in rhizotron system for 17 days. Both short- and long-term experiments were conducted in six controlled environmental growth chambers.

Experiment 2 deals with the effect of drought on peanut growth and *A. flavus* infection. This research work was investigated under both green house and open field conditions. In the green house experiment, contained 3 peanut genotypes, 4 water regimes, and 4 soil depths to periodic monitoring of root and *A. flavus* growth. A fast non-destructive method of observing root and *A. flavus* growth under soil by minirhizotron was used. Minirhizotron is a clear tube installed under soil surface that facilitates periodic root observation through the use of minirhizotron camera. Shoot and root growth were treated with different frequencies and fluctuating duration of water application, similar to conditions that occur naturally in semi-arid regions where rainfed peanut is produced.

Drought decreases yield quantity and quality of peanut. Low quality yield results from pre-harvest aflatoxin contamination. Drought especially during the later part of the growing season and temperatures from 25 to 38°C have been associated with enhanced levels of *A. flavus* infection in peanut (Holbrook *et al*., 1994a; Sanders *et al.*, 1993). If drought stressed plants are essential for *A. flavus* infection and subsequent aflatoxin contamination, then the mechanism for resistance could potentially involve some biochemical or physiological function of the plant. The results of biochemical responses of peanut pods to *A. flavus* infection under drought

conditions are revealed in an opened field experiment. This study used the same peanut genotypes as in the green house but differ in water treatments applied.

Experiment 3 studied infection pathways of *A. flavus* through peanut flowers. Strains of *A. flavus* that produced a green fluorescent protein (GFP) were used to track the pathways of infection which have not previously been clearly identified. Because *A. flavus* is largely a soil-borne fungus, thus in this experiment, three peanut genotypes were grown in half-Hoagland's nutrient solution to prevent the contamination from the soil surface.

A framework for research priority setting