Introduction

Aspergillus flavus and A. parasiticus are aflatoxin-producing species of Aspergillus that can infect peanuts and produce toxic effects in animals and humans. Aflatoxin B1, the most potent natural carcinogen known, and Aflatoxin B2 are described as carcinogenic, teratogenic, and immunosuppressive and are linked to serious liver damage (Scheidegger and Payne 2003; Bennett and Klich 2003). Pre-harvest infection occurs when peanut plants are under stress from environmental conditions or damaged by insects, and post-harvest infection can occur if peanuts are stored in conditions that favor mold growth. Detection of fungal infection, especially the aflatoxin-producing species of Aspergillus, is important before crops reach market. A thorough understanding of the host-pathogen interaction between peanuts and A. flavus or A. parasiticus may provide information that might be used to develop novel detection and screening methods for Georgia peanuts. The aim of this study is to establish the seed-borne nature of A. flavus and its significance in seedling infection using light microscopy, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).

Materials and methods

Raw peanuts purchased from retailers in Marietta, Kennesaw, and Cartersville were shelled and plated on moist filter paper. The plated seeds were incubated at 30°C for 5-7 days. Infestation by A. flavus was confirmed by light microscopy. Infected seeds, roots, and healthy seeds which served as controls, were processed for TEM and SEM using standard methods. Allsectioned samples were fixed in 3% gluteraldehyde overnight, secondary fixed overnight in 1% osmium tetroxide, followed by dehydration through a graded ethanol series. For TEM ethanol dehydration was followed by a final dehydration step in propylene oxide then infiltrated with Spurr’s epoxy resin (Cherry 1998; Flegler 1993; Xia 2000). The samples were then sectioned (70 to 90 nm), collected onto mesh copper grids, post stained with 5% uranyl acetate followed by Reynolds’ lead citrate stain and viewed with a JEOL 1210 LaB6 TEM. For SEM, dehydrated samples were placed in parafilm packets and cryofractured under liquid nitrogen. The fractured pieces were critical point dried with 2 as the transition fluid, mounted onto aluminum stubs and gold sputter coated. The coated samples were viewed in the lower stage of a DS-130 SEM with a LaB6 filament.

Two week old seedlings were also artificially infected with A. flavus spores, covered with polyethylene bags for 12 hours to provide 100% humidity and incubated at room temperature for seven days. Infected leaves were fixed in 70% ethanol, macerated, stained with methylene blue and viewed under light microscope.

Results

Results revealed dramatic differences between healthy and diseased tissues. The fungus established infection in the host tissues both intercellular and intracellularly aided by haustorium-like structures. While the fungal matrix contained a normal complement of cell organelles and several lipid droplets, cells of the invaded host tissues were irregular and some cells were completely destroyed. Moreover, cell wall lysis, granulation of cytoplasm, shrinkage of protoplasts and cell wall distortion and disruption were very common.

Our study also revealed that colonization of different parts of the seedling by A. flavus is aided by active and continuous branching (Fig. 3) of the young hyphae. Mycelium also colonized and established in the vascular tissue, mainly the xylem (Fig. 4). Cell walls of vascular tissue remained intact, but showed signs of severe disruption.

Conclusions

The toxic properties of the aflatoxin produced by Aspergillus flavus is a major concern for growers and consumers of peanut products. Elimination of the threat of infection is important before peanut seeds go into storage. The present investigation reveals that A. flavus is seed-borne and contaminated seeds are an important source of inoculum for seedling infection and spread of the fungus from one seed to another at storage and elsewhere. SEM and TEM proved to be a reliable method to detect the intercellular and intracellular hyphae of A. flavus, which is undetectable with the naked eye or by conventional light microscopy.

Literature cited


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Fig. 1. Healthy and seeds infected by A. flavus in peanuts (SEM 500x): a: Healthy seed with uninfected epidermis (E), well organized parenchyma (P) and uninterrupted cell walls (CW). b: Infected seed with fungal hyphae (H), rough epidermis (E) and disrupted parenchyma cells (P).

Fig. 2: Healthy and seeds infected by A. flavus in peanuts (TEM 3000x): a: Healthy seed tissues show abundant storage proteins (P) and smooth, well defined cell walls (CW). b: Infected seed shows disrupted cell walls and presence of intercellular (1) and intracellular hyphae (2). Bar 2µm

Fig. 3. Young hyphae of A. flavus inside the seed tissue in peanuts (4000x).

Fig. 4. Fungal hyphae (H) in xylem of peanut root. Intact and disrupted cell walls (CW).

Fig. 5. TEM (3000x) of epidermal cells in infected peanut seed. Intracellular hyphae (H) associated with destruction of cell wall (CW) and colonization of adjacent cell wall (P). Bar 2µm

Fig. 6. Infected peanut leaves (4000x). a. Necrotic tissue on cuticle layer and direct entry of the hyphae (H) into the stomata (S). b. Intracellular hyphae of A. flavus in leaf tissue.