CHAPTER 7

GENERAL DISCUSSION

It is well known that cowpea (*Vigna unguiculata* (L.) Walp) is considered to be a beneficial crop to cultivate and as a nutritious food crop it contributes positively towards food security to many rural communities living in less developed countries. This thesis highlights the potential problems and future prospects associated with this legume crop.

Although cowpea forms an essential part of cropping systems and nutrition in many countries of the tropics and sub-tropics (Singh *et al.* 2002), it is not regarded as a major crop contributing to household security in South Africa, in particular by rural communities of the Mpumalanga Province (Chapter 3). As predicted, the production of maize (*Zea mays* L.) ranked highest with regard to household security. Even though cowpea was grown predominantly for own consumption, the majority of the respondents ate cowpea less than once a week. The crop was used to a lesser extent as feed for livestock and as a source of income. In other countries, and especially in Central and West Africa, cowpea is utilised largely for all of these purposes (Singh *et al.* 2002). Interestingly, the survey indicated that 8.5% of the farmers used the crop for medicinal purposes. Generally, post-harvest storage problems were well perceived by the farmers and storage practices seemed adequate, with the exception of the use of harmful pesticides like DDT (dichloro diphenyl trichloroethane). However, 20% of the farmers encountered problems with fungal contamination during storage and in view of this, it is probable that contamination of the seed by mycotoxins could exist. Although the survey indicated that 60.6% of the farmers received training with regard to cowpea cultivation, relatively low percentages of the farmers practiced intercropping (53.5%) and crop rotation (40%).

An increasing awareness should be made amongst the farmers and rural communities of the advantages associated with cowpea production. These include the nutritional benefits as a food crop and the use of the plant in intercropping and crop rotation farming systems to increase soil fertility. The knowledge of the potential problems arising from fungal contamination of the seeds during storage and the harmful effects of mycotoxin ingestion should be disseminated to the farmers. This can be achieved through the continual interaction between the rural farmers and researchers, which has already been established. Other regions in South Africa where farmers cultivate cowpea should be surveyed in order to contribute additional information to the existing knowledge on the importance and role of cowpea in the livelihoods of rural communities.

As indicated in the literature review (Chapter 2) cowpea seed supports an extensive array of storage fungi and the presence of aflatoxin associated with cowpea seed has been established (El-Kady *et al.* 1996) thus presenting a potential health risk to consumers. In Chapter 4, cowpea seed samples from South Africa and Benin, West Africa were investigated for mycoflora infestation and fumonisin contamination. Amongst various fungal genera recorded, including *Aspergillus*, *Phoma* and *Lasiodiplodia*, the results indicated an array of *Fusarium* spp. including *F. equiseti* (Corda) Sacc., *F. chlamydosporum* Wollenweber and Reinking, *F. graminearum* Schwabe, *F. sambucinum* Fuckel, *F. scirpi* Lamb. et Fautr., *F. semitectum* Berkeley & Ravenel and *F. subglutinans* (Wollenw. and Reink.) Nelson, Toussoun, and Marasas. Four isolates of *F. proliferatum* (Matsushima) Nirenberg were isolated from the seeds. The cowpea cultivars from South Africa showed the presence of FB_1 with concentrations ranging between 0.12 - 0.61 µg/g. Fumonisin B_1, B_2 and B_3 were produced by all *F. proliferatum* isolates. Total fumonisin concentrations were between 0.80 - 25.30 µg/g and the highest level of FB_1 detected was 16.86 µg/g. This was lower than FB_1 levels produced by *F. proliferatum* isolates isolated from the seed of other crops (Ross *et al.* 1990; Thiel *et al.* 1991). These studies represent the first report of the natural occurrence of FB_1 on cowpea seed and of the potential of *F. proliferatum* isolates from cowpea seed to produce fumonisin mycotoxins. In light of the substantial use of cowpea seed for food by people in many tropical and sub-tropical countries, the susceptibility of cowpea to *Fusarium* infection and fumonisin contamination remains a potential health hazard. Further screening for fumonisins is necessary to establish the degree of fumonisin contamination in cowpea seeds. Further studies on the other mycoflora associated with the seed are needed since some of the fungi found during these investigations, including *Aspergillus flavus* Link ex. Fries, *F. equiseti*, *F. graminearum*, *F. sambucinum* and various *Penicillium* spp. are known to produce other mycotoxins of toxicological significance (Desjardins & Hohn 1997).

Given the occurrence of FB_1 on cowpea seeds and the phytotoxic accounts relating to FB_1 (Abbas & Boyette 1992; Doehlert *et al.* 1994; McClean 1996), it was found necessary to investigate the effects of the toxin on cowpea seed (Chapter 5). Cowpea seeds were treated with purified FB_1 at concentrations of 10, 25, 50 and 100 ppm. The results indicated that all the FB_1 concentrations significantly decreased seed germination. This complimented the findings of Danielsen & Jensen (1998) where a significant negative correlation was found between fumonisin content and maize seed germination. On the other hand Doehlert *et al.* (1994) reported that FB_1 had no effect on maize seed germination. Known FB_1 producing strains of *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum*, which were inoculated onto the seeds also showed a significant reduction in seed germination. Further studies are required to establish if this was a result of fungal infestation alone or of both fungal infestation and the production of a toxin. This study also supported findings from other studies (Doehlert *et al.* 1994; Lamprecht *et al.* 1994) that FB_1 plays an inhibitory role in root and shoot growth of plants. Cowpea root and shoot elongation were significantly inhibited by the 50 and 100 ppm FB_1 concentrations and apparent physical abnormalities were seen amongst these seedlings. In an attempt to understand the actions of FB_1 at a cellular level, the ultrastructural changes caused by the toxin were studied by means of transmission electron microscopy (TEM). Damaging effects by FB_1 were only noted in the seeds treated with the highest concentration of toxin (100 ppm). These included the contraction of the protoplasm, which resulted in the formation of numerous vacuoles within the much more dense protoplasm. The plasma membrane also separated from the cell wall. These findings constitute the first report of the phytotoxic effects of FB_1 on cowpea seed.

Fumonisin B_1 interferes with the synthesis of sphingolipids through the inhibition of the enzyme, ceramide synthase (Riley *et al.* 1994). Sphingolipids are important components of cell membranes and play an important role in cell regulation, membrane stability and stress response in plants (Riley *et al.* 1994; Sperling & Heinz 2003). The interference in their metabolism results in disturbances in growth, differentiation and morphology of cells (Riley *et al.* 1994). This disruption of sphingolipids is a probable explanation for the destructive effects noticed in the seed tissue. However, further research is necessary to determine the precise toxic effects of the toxin in order to understand why seed germination and root and shoot growth of the seedlings were adversely affected.

As indicated from findings from this study and from other reports (van Wyk & Gericke 2000), cowpea is used for medicinal purposes. To provide the foundation from which to pursue the scientific substantiation of these reports, the antimicrobial activity of leaf extracts of two cowpea cultivars were evaluated (Chapter 6). The results indicated that the acetone and ethanol extracts of the leaves of both cowpea cultivars (Bechwana White (BW) and Kpodjiguégué (Kpod), significantly inhibited the growth of all the fungal plant pathogens at 5.0 mg/ml, with the exception of *F. equiseti*. *Alternaria alternata* (Fr: Fr.) Keissler was further inhibited by the acetone extract of Kpod and both BW extracts at 2.5 mg/ml. *Fusarium proliferatum* was inhibited by the BW ethanol extract at 2.5 mg/ml. Antibacterial activity was predominantly displayed by the BW acetone extract. A minimum inhibitory concentration (MIC) of 2.5 mg/ml was recorded for *Staphylococcus aureus* Rosenbach and *Enterococcus faecalis* (Andrews and Horder) Schleifer and Kilpper-Balz whilst *Bacillus cereus* Frankland and Frankland, *B. subtilis* (Ehrenberg) Cohn and *Enterobacter cloacae* (Jordan) Hormaeche and Edwards were inhibited at a concentration of 5.0 mg/ml. The BW ethanol extracts inhibited the growth of *E. faecalis* and *E. cloacae* at 5.0 mg/ml. This is the first report on the inhibitory effect of cowpea leaf extracts on the growth of human bacterial and fungal plant pathogens. It is concluded from this investigation that the crude extracts from cowpea be further fractionated in order to purify and identify the active compounds responsible for antimicrobial inhibition.

The results presented in this thesis indicate the challenges facing future research on cowpea. The use of cowpea seed as a source of nutritious food can be hampered through fungal infestation and subsequent fumonisin contamination of the seed. Further research is imperative to establish the extent of this potential problem, especially amongst rural communities where storage practices are often inadequate. Not only does FB_1 contamination pose a threat to human and animal health, but it also holds a negative impact for the plant itself. The toxin could impede cowpea seed germination and the ability to emerge from the ground during germination in the field. This can be investigated through experiments in the glasshouse and in the field. Further investigation into the antimicrobial compounds associated with cowpea extracts will enhance the existing benefits of the crop.

The value of cowpea in sub-Saharan Africa can be amplified through the effective prevention and control of storage fungi and subsequent mycotoxin contamination, and through the exploitation of the beneficial compounds in the plant as potential alternative sources of medicine.

7.1. LITERATURE CITED

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SUMMARY

Cowpea is an indigenous African legume crop that is of great importance to the livelihood of many subsistence farmers and rural communities in tropical and subtropical countries. This resourceful legume crop is used as a nutritious source of food by people, as fodder for livestock, as a means to generate income through the trade of the seed and for medicinal purposes.

Cowpea was ranked third against maize and vegetable production with regard to the importance to the livelihoods of farmers surveyed in the Mpumalanga Province of South Africa. The crop was mainly grown for own consumption, with the purposes as a source of income and feed for livestock to a lesser extent. Some of the farmers used the roots and seeds for medicinal purposes. Although the farmers practiced adequate post-harvest practices, mouldiness of the seed was reported by 20% of the farmers with insect infestation to a lesser extent.

Cowpea seeds of seven cultivars (four from South Africa and three from Benin, West Africa) were analysed for seed mycoflora and fumonisin contamination. *Aspergillus*, *Phoma* and *Lasiodiplodia* spp. where predominantly isolated from these seed samples. The results indicated low percentages of *Fusarium* spp., including *F. equiseti*, *F. chlamydosporum, F. graminearum, F. sambucinum*, *F. semitectum*, *F. subglutinans* and *F. proliferatum*. The seed samples and *F. proliferatum* isolates, cultured on maize patty media, were extracted with methanol/water and cleaned-up on strong anion exchange solid phase extraction cartridges. Fumonisins were quantified as *o*-phthaldialdehyde pre-column derivatives by high performance liquid chromatography (HPLC) and fluorescence detection. Fumonisin B_1 (FB_1) was present in four seed samples with a range of 0.12 - 0.61 µg/g. The results revealed that all the *F. proliferatum* isolates produced FB_1, FB_2 and FB_3. Total fumonisins ranged between 0.80 - 25.30 µg/g and the highest FB_1 level was 16.86 µg/g.

In order to investigate the phytotoxic effects of FB_1 on cowpea seed, surface disinfected cowpea seeds were imbibed in sterile water amended with pure FB_1 to yield final concentrations of 10, 25, 50, and 100 ppm. Percentage germination, as determined according to the International Seed Testing Association (ISTA) rules, was significantly decreased at all toxin concentrations. Root and shoot length was measured after 9 days and the 50 and 100 ppm toxin concentrations showed an inhibitory effect on their growth. Embryonic axes and cotyledon tissues were removed and prepared for transmission electron microscopy (TEM) studies. Ultrastructural deteriorations included compaction of the protoplasm and separation of the plasmalemma from the cell wall. Furthermore, lipid bodies accumulated and lined the cell wall.

Acetone and ethanol extracts of the leaves of the Bechwana White and Kpodjiguégué cultivars of cowpea were investigated for their antimicrobial properties against human bacterial and fungal plant pathogens. All the extracts significantly inhibited the growth of all the fungal pathogens at 5.0 mg/ml, with the exception of *Fusarium equiseti*. Growth of *Alternaria alternata* and *F. proliferatum* was significantly reduced by certain extracts at 2.5 mg/ml. Acetone extracts of Bechwana white inhibited the growth of *Staphylococcus aureus* and *Enterococcus faecalis* at 2.5 mg/ml and *Bacillus cereus*, *B. subtilis* and *Enterobacter cloacae* at 5.0 mg/ml whilst the ethanol extracts of the same cultivar only showed antibacterial activity against *E. faecalis* and *E. cloacae* at 5.0 mg/ml.

This study is the first report of the natural occurrence of fumonisins on cowpea seed and of the production of fumonisins by *F. proliferatum* isolates isolated from cowpea seed. Furthermore, reports of the phytotoxic effects of FB_1 on cowpea seeds and the antimicrobial activity of cowpea leaf extracts were established.