

CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND AND MOTIVATION OF THE STUDY

Cowpea (*Vigna unguiculata* (L.) Walp) is an indigenous African legume crop that is widely cultivated in the semi-arid tropical regions of Asia, Oceania, Africa, the Middle East, southern United States of America and Central and South America (Singh *et al.* 2002). In South Africa, 7000 t of cowpeas was produced from 13,500 ha during 2003 (FAOSTAT 2004). This production figure compares weakly with other legume crops including soybeans (*Glycine max* L.), groundnuts (*Arachis hypogaea* L.) green peas (*Pisum sativum* L.) and lupines (*Lupinus* spp.) (Table 1.1.).

Table 1.1. Production figures of different grain, pulse and oilseed crops in South Africa for 2003 (FAOSTAT 2004)

Commodities	Production (t) for 2003
Maize (<i>Zea mays</i> L.)	9,714,254
Wheat (<i>Triticum aestivum</i> L.)	1,600,000
Sunflower (<i>Helianthus annuus</i> L.) seed	708,000
Sorghum (<i>Sorghum bicolor</i> (L.) Moench.)	224,818
Barley (<i>Hordeum vulgare</i> L.)	150,000
Soybeans (<i>Glycine max</i> L.)	148,000
Groundnuts (<i>Arachis hypogaea</i> L.)	100,000
Oats (<i>Avena sativa</i> L.)	35,000
Peas (<i>Pisum sativum</i> L.), green	18,545
Millet (<i>Pennisetum glaucum</i> (L.) K Schum.)	12,000
Lupines (<i>Lupinus</i> spp.)	11,700
Cowpeas (<i>Vigna unguiculata</i> (L.) Walp)	7,000
Rice (<i>Oryza sativa</i> L.)	3,200
Peas, dry	1,033
Rye (<i>Secale cereale</i> L.)	550

Cowpea is of particular importance to many people living in less developed countries, where resource poor farmers and rural communities rely largely on the crop as a source of nutritious food. The seed contains on average 23-25% protein and 50-67% starch (Quin 1997). The young leaves, green pods and green seeds are used as vegetables whilst the dry seeds are used in the preparation of various food dishes (Singh *et al.* 2002). The haulms are also used as a quality feed for livestock (Singh *et al.* 2002). The plant is well adapted to hot and dry climates, has the ability to fix atmospheric nitrogen, curbs soil erosion through good ground cover and contributes to soil fertility through decayed residues (Singh *et al.* 2002). Furthermore, the trade of the fresh produce, seeds and foods processed from cowpea provides many farmers with a source of income (Singh *et al.* 2002). Medicinally, the leaves are used to treat burns, a root paste is used as an antidote for snake bites whilst the seeds are used to treat bilharzia, liver complaints and amenorrhoea (Hutchings *et al.* 1996; van Wyk & Gericke 2000). Previous studies have shown that cowpea leaves contain flavonoids (Lattanzio *et al.* 1997) of which some are known to exhibit antimicrobial properties (Aziz *et al.* 1998). However, the direct influence of cowpea extracts on microbes still remains to be established.

As is the case with other food crops, cowpea seeds are susceptible to fungal contamination especially when stored under inadequate and poor storage conditions. As a result, deterioration and spoilage of the seed occurs. It is under these conditions, together with high humidities and high temperatures that certain fungi tend to produce toxic secondary metabolites, namely mycotoxins. Serious health complications in both animals and humans can result from the ingestion of these metabolites through the consumption of infected seed and other foodstuffs (Moss 1996). Although there are reports relating to aflatoxin contamination (Zohri 1993; El-Kady *et al.* 1996), little information is known about other mycotoxins associated with cowpea seed.

Fumonisin, and in particular the analogue fumonisin B₁ (FB₁), is considered to be of major toxicological significance to animal and human health (Shephard *et al.* 1996). This toxin is produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg (formerly known as *F. moniliforme* Sheldon) and *F. proliferatum* (Matsushima) Nirenberg and is the causal agent of the diseases, leukoencephalomalacia (LEM) in horses and pulmonary edema syndrome (PES) in pigs (Harrison *et al.* 1990; Kellerman *et al.* 1990). The International Agency for Research on Cancer (IARC) reported that the toxins produced by *F. verticillioides* are possibly carcinogenic to humans (IARC 2002). Fumonisin B₁ has been reported to be associated with other legume seeds (Tseng *et al.* 1995; Tseng & Tu 1997), but the *Fusarium* spp. responsible for the toxin production were not identified.

Numerous studies have indicated that FB₁ also exhibits phytotoxic activity towards several plants, including economically important crops (McClellan 1996). The toxin is reported to cause deleterious

effects in various crops including chlorosis and necrosis of leaves and reduction of root and shoot growth (Abbas & Boyette 1992; Doehlert *et al.* 1994; Lamprecht *et al.* 1994). Moreover, van Asch (1990) showed that FB₁ caused ultrastructural changes in maize (*Zea mays* L.) callus cells. These changes included thickening of the cell wall, the accumulation of phenolics in the vacuoles and accumulation of large starch grains in swollen plastids.

1.2. OBJECTIVES OF THE STUDY

The primary objectives of this study were firstly to investigate the presence and effects of fumonisins associated with cowpea seed and, secondly to evaluate the antimicrobial activity of the leaf extracts. The results that stem from these studies will provide valuable information with regard to further mycotoxin contamination of cowpea seed and the potential use of the plant extracts for antimicrobial purposes.

The specific objectives of this study were to:

- ❖ Conduct a survey amongst rural communities in the Mpumalanga Province of South Africa to establish the importance, role and utilisation of cowpea production in this region.
- ❖ Investigate the detection and quantification of the fumonisin mycotoxins in cowpea seeds.
- ❖ Identify the fumonisin-producing *Fusarium* spp. from cowpea seeds and to investigate their potential for fumonisin production.
- ❖ Investigate the phytotoxic effects of FB₁ on cowpea seed. These include the effect on germination, root and shoot elongation and on the ultrastructure of the cotyledon and embryonic tissue of the seed.
- ❖ Investigate the inhibitory effect of cowpea leaf extracts on the growth of selected bacterial and fungal pathogens.

1.3. STRUCTURE OF THESIS

Some of the chapters presented in this thesis have been prepared for publication in peer-reviewed journals. The reader will thus note some inconsistencies within the contents page and format of the chapters. Author citation and literature citation in Chapters 1, 2, 3 and 7 are according to the South African Journal of Botany.

- Chapter 2** This chapter provides a concise review of the fungi and mycotoxins associated with cowpea seed. Information has been given on the fumonisin mycotoxins and their toxicological significance. The use of plant extracts for antimicrobial activity and secondary metabolites associated with cowpea have also been briefly discussed.
- Chapter 3** The importance and role of cowpea in the livelihoods of rural communities was established by means of a survey conducted in the Mpumalanga Province of South Africa. The survey was aimed to gather information on the utilisation of the crop and to provide insight on the cultivation and storage practices followed.
- Chapter 4** Cowpea seeds were analysed for storage fungi and fumonisin contamination. Possible fumonisin-producing *Fusarium* spp. isolated were investigated for potential to produce fumonisins.
Published as: Kritzinger Q, Aveling TAS, Marasas WFO, Rheeder JP, van der Westhuizen L, Shephard GS. (2003) Mycoflora and fumonisin mycotoxins associated with cowpea (*Vigna unguiculata* (L.) Walp) seeds. *Journal of Agricultural and Food Chemistry* **51**: 2188-2192.
- Chapter 5** The phytotoxic effects of FB₁ on cowpea seed germination and on root and shoot elongation were investigated. The effect of the toxin on the ultrastructure of the cotyledon and embryonic tissue of the seed was also reported. (Submitted to *Phytopathology* for publication)
- Chapter 6** Leaf extracts from two cowpea cultivars were tested for their antimicrobial potential against selected bacterial and fungal pathogens.
In press as: Kritzinger Q, Lall N, Aveling TAS (2004) Antimicrobial activity of cowpea (*Vigna unguiculata*) leaf extracts. *South African Journal of Botany*.
- Chapter 7** In this chapter, the findings from the research conducted have been interpreted and discussed, and suggestions for future research made.

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