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Biological and chemical control of fungal seedling diseases of cowpea

By

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Submitted in partial fulfillment of the requirements for the degree of

M.Inst. Agrar. (Plant Protection)
In the Faculty of Natural and Agricultural Sciences
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University of Pretoria

Pretoria

March 2006

DECLARATION

I declare that the dissertation herewith submitted for the degree of M.Inst. Agrar (Plant Protection) at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education

……..

Ramusi Tshekgene Moses

ACKNOWLEDGEMENTS

The author would like to thank the following people who made valuable contributions to the completion of his thesis:

The Almighty God for giving me strength for the completion of this thesis, without whom none of this would have been possible.

My supervisor Prof T. Aveling and co-supervisors Prof N. Labuschagne and Doctor J. van der Waals for their valuable scientific advice, criticism, support and encouragement.

My parents Melitah for her encouragement, support and love and the late Moshweu, may his soul rest in peace.

All my sisters and brothers especially Gertrude for her financial support, encouragement and everlasting love throughout all my studies.

My deep gratitude goes to my friends Sydney Mello and Hellen Ramaloko for their excellent friendship and always being there to give valuable support, encouragement and everlasting love.

All people in the department of Microbiology and Plant Pathology especially lab 2-12 for their everlasting love, support and encouragement.

My pastor Rakgabale for his valuable encouragement, support and always being there to listen to my problems.

The NRF for financing this study.

All my friends who gave valuable contribution that led to the completion of this thesis.

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SUMMARY

Cowpea is a worldwide-distributed crop, and is important to the livelihood of poor people in developing countries. Cowpea is also susceptible to a wide range of pests and pathogens, which can cause damage to the crop at all stages. Seedling diseases caused by pathogens such as *Rhizoctonia solani*, *Fusarium solani* and *Pythium* spp. affect cowpea, and result in low yields, especially in rural areas where there are few or no control measures against these pathogens. This research aimed at evaluating the efficacy of a biological control agent and fungicides against fungal seedling diseases of cowpea.

The bacterium, *Bacillus cereus1*, and the fungicides, Apron®, Subdue® and Celest® were screened for the control of cowpea seedling diseases, after obtaining promising *in vitro* results on their effectivity against *Rhizoctonia solani, Pythium ultimum* and *Fusarium solani*. The experiment was conducted in a greenhouse using seedling trays with 128 cells, each filled with pasteurised growing medium (Braaks lawn dressing). Seedling trays were placed randomly on greenhouse tables with four replication per treatment, each replication consisting of 56 plants. Cowpea seeds (Cultivar-Pietersburg blue) were obtained from the Dry Bean Seeds Producers Organisation.

The pasteurised growing medium was artificially inoculated with the three fungi. Two plugs of actively growing fungal mycelium of the three pathogens were inoculated in each cell of the polystyrene seedling trays. Trays were drenched with *Bacillus cereus1* at 10^6 cells/ml (3 ml per tray cell) at planting and fungicides were applied on the 14^{th} and 28^{th} days at the recommended rate. The experiment was conducted at temperatures ranging from 22-25 ^oC. Plants were harvested on the 35^{th} day after planting and percentage germination, diseased, height of the plants and dry mass of roots and shoots were determined.

Results indicated that the biological control agent (*B. cereus1*) was able to significantly reduce the damage done by the pathogens *Rhizoctonia solani, Pythium ultimum* and *Fusarium solani* in all trials. It was also confirmed that the application of the biological control agent during planting could reduce disease incidence. The biological control agent increased seed emergence rate and shoot length.

All three fungicides significantly reduced the disease incidence caused by all pathogens. All fungicides treatments applied increased emergence rate and shoot length.

Seedling diseases should be given too much attention, as they cause severe losses to many crops. There is a need for future research on the effectivity of *B. cereus1* as relatively little work has been published on its antagonistic behaviour against seedling diseases. There are also few registered fungicides available for the control of these seedling diseases on cowpea, therefore research on these and other potential products is required as seedling diseases play a major role in reducing yield of many crops.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Cowpea is important for the livelihood of poor people in undeveloped countries, and can be used for various purposes such as a food crop, cash crop, and animal feed (Singh *et al*., 1997). It is considered the most economically important traditional legume crop in Africa (Langyintuo *et al*., 2003). Cowpea is distributed worldwide especially in arid and semi arid areas (Zohri *et al*., 1992).

Cowpea is susceptible to a wide range of pests and pathogens, which can cause damage to the crop at all stages of growth (Summerfield & Roberts, 1985). Seedling diseases in cowpea result in low yields, especially in rural areas where no control measures are taken against the diseases. In countries such as Nigeria, seedling diseases caused by *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp. are of economic importance and they can cause great losses in the low altitude rain forests because of seed decay and seedling damping-off (Singh & Rachie, 1985). Farmers in poor developing countries are unable to control the diseases because of financial constraints.

1.2 Motivation for the study

Cowpea is susceptible to most pathogens that attack legumes, including root rot and damping-off. Complete eradication of the pathogen causing root rot and damping-off in cowpea is difficult (Davis *et al*., 1991; Valenzuela and Smith, 2002). The International Institute of Tropical Agriculture has reported that stem and root rots are considered the major diseases of cowpea. Edema *et al*. (1997), in their study focusing on cowpea diseases in Uganda, found that most farmers (approximately 94 %) have no control strategies for cowpea diseases.

According to research done by Isubikalu *et al.* (1999), it was found that most farmers have some knowledge on alternative methods for controlling pests but they have limited knowledge on measures available for controlling cowpea diseases. They also found that the main means currently available for controlling cowpea pests is by using pesticides which are expensive. A previous study by Davis *et al.* (1991) indicated that there is a need for the development of some alternative measures for the control of cowpea diseases. Therefore, this project is aimed at finding control measures, which may be effective in controlling seedling diseases of cowpea.

1.3 Aim of the study

The aim of this study was to evaluate the efficacy of a biological control agent and fungicides against fungal seedling diseases (*Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani* Kühn and *Pythium ultimum* var *ultimum* Trow) of cowpea.

1.4 Objectives

-Evaluate the efficacy of fungicides and a biological control agent against fungal seedling diseases of cowpea.
-Find an alternative control measure (biological control agent), which is effective and affordable.
-Determine what effects the various treatments have on cowpea seedling emergence and growth.

CHAPTER 2

LITERATURE REVIEW

2.1 Cowpea (*Vigna unguiculata* L. Walp.)

2.1.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is the most economically significant African traditional legume crop, which is important for poor people in less developed countries (Valenzuela & Smith, 2002; Langyintuo *et al*., 2003). Cowpea is referred to as southern pea, blackeye pea, crowder pea, lubia, niebe, cowpea or frijole (Davis *et al*., 1991). Marechal *et al*. (1978) reclassified the subspecies *unguiculata*, *catjang* and *sesquipedalis* as cultigroups *Unguiculata*, *Biflora*, and *Sesquipedalis* and grouped them under *V. unguiculata*, as reported by Davis *et al*. (1991). According to Singh *et al*. (1997), cowpea is a dicotyledon classified in the family *Fabaceae*, subfamily *Faboideae*, tribe *Phaseolinae*, order *Fabales*, section *Catiang*, and genus *Vigna*.

Cowpea is distributed worldwide, especially in arid and semi arid areas (Zohri *et al*., 1992). Cowpea is also significant for the livelihood of poor people in undeveloped countries because it has multiple uses such as for food, as a cash crop and as animal feed (Singh *et al*., 1997). It is a source of high quality protein and a cash crop for most West and Central African farmers (Langyintuo *et al*., 2003). According to Phillips *et al*. (2003), cowpea is also considered a significant component of diets in developing countries of Africa, Latin America and Asia where it is used as a dietary protein to complement cereals.

Cowpea can be grown under various production systems such as rain-fed, irrigated, and in areas of poor soil and low rainfall (Singh *et al*., 1997). According to Singh *et al*. (1997), cowpea can be used for intercropping with sorghum and millet and groundnuts. The intercropping of cowpea is significant in controlling soil erosion and weeds (Singh *et al*., 1997).

Cowpea is susceptible to a wide range of pests and pathogens, which can cause damage to the crop at all stages of growth (Summerfield & Roberts, 1985). Diseases of cowpea are induced by viruses, fungi, nematodes, parasitic flowering plants and adverse environmental factors such as temperature and relative humidity (Davis *et al*., 1991)

2.1.2 Origin, taxonomy and distribution

Common cowpea, *Vigna unguiculata*, was first domesticated in Africa (Singh & Rachie, 1985; Davis *et al*., 1991). Five sub-species of *V. unguiculata* are recognized, which are: ssp. *unguiculata*, the cowpea; ssp. *cylindrica*, the catjang; ssp. *sesquipedalis*, the yard-long or asparagus bean; ssp. *dekindtiana* and ssp. *mensensis*, the progenitors of the cultivars (Allen, 1983).

The northern region of South Africa was established as the centre of origin of *V. unguiculata*, due to the availability of most primitive wild varieties, e.g. var. *rhomboidea*, var. *protracta*, var. *tenuis* and var. *stenophylla* (Singh *et al*., 1997). From the northern regions of South Africa, the species was dispersed to Mozambique and Tanzania where it evolved into two subspecies, (*tenuis* sp. and *staphylla* sp.) which share similar eco-geographical distribution from South Africa to Zimbabwe and Mozambique (Singh *et al*., 1997).

Variety *congolensis* is found in the Congo basin and var. *huillensis* in the savannah regions across Namibia and Miombo in Southern Africa; whereas var. *ciliolate* appears in the forest areas of Burundi, Malawi, Zambia, Zimbabwe, the south western Cape flora of South Africa and in the eastern Kivu region of Zaire (Singh *et al*., 1997). Variety *dekindtiana* is dispersed throughout Africa, south of the Sahara including Madagascar and it is believed to be the progenitor of the cultivated cowpea (Singh & Rachie, 1985).

In West and Central Africa cowpea cultivation covers more than eight million hectares of which Nigeria is the largest producer at four million ha, followed by Niger with three million ha (Singh & Eaglesfield, 2000). In east Africa, Tanzania and Mozambique are the major informal cowpea exporters, while Malawi, Zambia,

Uganda, Kenya and Democratic Republic of Congo are major importers of cowpea (Tchale, 2001).

Cowpea is a warm-season crop adapted to many areas of the humid tropics and temperate zones and can tolerate drought (Davis *et al*., 1991). Cowpea performs best on well-drained sandy loams or sandy soils where soil pH ranges from 5.5 to 6.5 (Davis *et al*., 1991) and can grow well in temperatures ranging from 20 to 35 ºC (Valenzuela & Smith, 2002). Cowpea can be grown under both irrigated and non-irrigated conditions, however, it does not withstand waterlogged or flooded conditions (Valenzuela & Smith, 2002).

2.1.3 Uses of cowpea

The major producers and consumers of cowpea are subsistence farmers in the semi-arid and sub-humid regions of Africa (Fery, 2002). Cowpea is a nutritious crop that contains proteins, vitamins and minerals (Table 2.1) (Singh *et al*., 1997; Taiwo, 1998; Fred, 2002; Egounlety, 2002). Leaves, immature pods and peas are used as vegetables, and grains are used for several purposes such as snacks and main meals (Singh *et al*., 1997). Cowpea can also be used as a forage or cover crop to suppress weeds, control erosion, and attract beneficial insects (Summerfield & Roberts, 1985; Lal, 1998; Valenzuela & Smith, 2002). Young shoots can be boiled and eaten as spinach. In some countries, such as Nigeria, mature leaves are boiled and dried in the sun to be used as a relish, when fresh vegetables are scarce.

Cowpea paste, prepared from dried peas, is the primary ingredient for the well-known Nigerian fried cowpea product “Akara” (Bulgarelli *et al*., 1988). When preparing paste, seeds are stored in water, manually decorticated, ground to a paste and the paste then whipped to incorporate enough air into the mixture to facilitate the formation of a stable foam (Singh & Rachie, 1985).

Cowpea seeds can also be processed into composite flour for baking applications, Akara (West African finger food made from soaked, decorticated, wet-millet cowpeas), extruded snacks, weaning foods (as an ingredient in prepared food for

weaning children especially those in transition from breast milk to solid food), or for traditional foods (Ehlers & Hail, 1997; Taiwo, 1998; Phillips *et al*., 2003).

Table 2.1 Nutrient content of mature cowpea seeds (Singh & Rachie, 1985)

Protein	24.8 %
Fats	1.9 %
Fibre	6.3 %
Carbohydrates	63.6 %
Thiamine	0.00074 %
Riboflavin	0.00042 %
Niacin	0.00281 %

According to Singh *et al*. (1997) and Fred (2002), an important characteristic of cowpea is that it plays a major role in the fixation of nitrogen through symbiosis with nodule bacteria (*Bradyrhizobium* spp.). Cowpeas also increase soil organic matter content and improvement of soil structure after soil incorporation (Valenzuela & Smith, 2002). Fibre of cowpea is used to make fishing lines, and has also been considered as a source of pulp to make good quality paper (Summerfield & Roberts, 1985).

The hulls of cowpea are highly digestible and can be used as feed for livestock and are therefore significant for farmers (Savadogo *et al*., 2000; Ajeigbe *et al*., 2003). The use of cowpea as a dual-purpose crop, which provides both grain and fodder, is attractive in mixed cropping systems (crop and livestock) where land and feed are scarce (Singh *et al*., 1997).

Cowpea animal fodder has higher nutritive values than many leguminous crops (Inaizumi *et al*., 1999). Cowpea also contributes to the sustainability of cropping systems and soil fertility improvements in marginal lands by providing ground cover and plant residues, and suppressing weeds (Davis *et al*., 1991; Valenzuela & Smith, 2002).

2.1.4 Pests and diseases of cowpea

Cowpea is susceptible to a wide range of pests and pathogens, which attack the crop at all growth stages, and pose a serious threat to production in South Africa, since cowpea is now being grown in greater abundance and in monoculture (Aveling *et al*., 2001). Cowpea is a hardy crop that harbours many pests, including leafminers, whiteflies, leafhoppers, mites, thrips, and aphids (Valenzuela & Smith, 2002). The greatest losses in cowpea yield occur in the low-altitude rain forests because of seed decay and seedling damping-off (Singh & Rachie, 1985).

The most significant and widespread cowpea diseases are caused by pathogens such as rust (*Uromyces phaseoli* var. *vignae*. Barclay), bacterial canker (*Xanthomonas vignicola* [Burkholder] Dye), *Fusarium* wilt (*Fusarium oxysporum* Schl. f.sp. tracheiphilum), powdery mildew (*Erysiphe polygoni* DC.), anthracnose (*Colletotrichum gloeosporioides*) (Penz.) Penz, anthracnose (*Colletotrichum lindemuthianum* [Sacc and Magn.] Briosi and Cav.), and viruses such as yellow mosaic, green mottle and tobacco mosaic virus (Singh *et al*., 1997). *Pythium*, *Rhizoctonia* and *Fusarium* result in root rot and damping-off in cowpea and their symptoms vary, which include rapid death of young succulent plants, stunting, wilting and cracking of the stem (Davis *et al*., 1991).

2.2 Major fungal diseases

2.2.1 *Pythium* soft stem rot

Pythium spp. are considered significant in warm, humid tropical conditions such as those of the rainforest, the southern part of Southern Guinea, the savannah of West and Central Africa and humid, subtropical zones of India, because of the damage they cause to crops (Singh *et al*., 1997).

Pythium soft stem rot is characterised by a grey-green, water-soaked girdle of the stem extending from the soil and including the lower branches (Singh & Rachie 1985; Davis *et al*., 1991). According to Singh *et al*. (1997), the slimy stem base is covered by white, cottony mycelial growth during periods of high humidity. The disease

incidence is increased with high plant populations, while use of average plant populations can lower the infection rate (Singh *et al.*, 1997). Some fungicides give better disease control when used as a seed treatment than a soil drench (Singh *et al.*, 1997).

2.2.2 *Fusarium* wilt

Symptoms of *Fusarium* wilt (*Fusarium oxysporum* Schl. f.sp. *tracheiphilum* (E.F. Smith) Snyder and Hansen) include stunting of the affected cowpea plant, chlorosis, dropping, premature defoliation, withering of leaves and brownish purple discolouration of vascular tissues (Singh & Rachie, 1985; Davis *et al*., 1991; Boyhan *et al.*, 1999). The leaves become flaccid and chlorotic, and young plants show fairly rapid wilting leading to death. Transmission occurs through soil and probably seed (Singh *et al.*, 1997).

The disease can be prevented by using resistant cowpea varieties (Singh & Rachie, 1985). Root knot nematodes provide conducive conditions for the pathogen to infect the plant, therefore their control will help in reducing the rate of infection by *Fusarium* (Davis *et al*., 1991).

2.2.3 *Rhizoctonia* diseases

Rhizoctonia solani Kühn is a soil-borne pathogen that causes stem canker, storage rot, aerial blight, and seedling damping-off diseases in many crops such as cowpea, soybean, carrots and potato (Carisse *et al.*, 2001). *Rhizoctonia bataticola* [(Taub.) Butler] causes a seedling disease of cowpea that is commonly known as charcoal rot. The pathogen overwinters as sclerotia under adverse soil environmental conditions (Carisse *et al.*, 2001).

Fungicides in combination with other chemicals can control *R. solani* (Singh & Rachie, 1985). Use of biological control agents such as endophytic bacteria and fungal antagonists including *Trichoderma* spp. and *Gliocladium virens* Miller, Giddens and Foster have shown potential for practical applications in agriculture (Carisse *et al*., 2001).

2.2.4 Anthracnose

Anthracnose of cowpea is caused by the pathogen *Colletotrichum gloeosporioides* f.sp. *aeschynomene* (CGA) (Singh *et al*., 1997). The pathogen attacks the stem, leaves and pods (Boyhan *et al*., 1999). Symptoms are brown, sunken and lenticular lesions that expand quickly and coalesce to girdle stems, peduncles and petioles on susceptible species of cowpea (Allen, 1983; Smith *et al*., 1997). The primary source of inoculum is seed and secondary sources are rain splash, air currents and contact with man and animals (Singh & Rachie, 1985). Wet and humid conditions during the growing season are favourable for anthracnose (Singh *et al*., 1997). The severity of the disease can also be increased by a high plant population (Edema *et al*. 1997).

According to Singh *et al*. (1997), the use of resistant varieties controls anthracnose diseases. Use of foliar fungicides such as benomyl and carbendazim can reduce epidemics by 40 to 45% (Singh *et al*., 1997). Some strains of *Colletotrichum* species with resistance to fungicides such as carbendazim and thiophanate-methyl have been discovered in India (Singh *et al*., 1997). Alcohol and water extracts of *Piper nigrum* L. Query IPNI, *Ocimum sanctum* L. and *Citrus limon* (L.) Burm are considered to be effective in reducing diseases of *Colletotrichum* spp. of cowpea *in vitro* and *in vivo* as reported by Amadioha (2003).

2.2.5 *Ascochyta* blight

Ascohyta phaseolorum Sacc. causes a seed-borne disease in cowpea (Allen, 1983). Symptoms are severe defoliation and lesions on the stems and pods, which can result in death (Singh *et al*., 1997). The pathogen overwinters in infected debris and in certain perennial hosts (Allen, 1983; Emechebe & Florini, 1997). Primary inoculum is seed and plant debris and secondary inoculum is rain splash, air currents and wind driven moisture (Emechebe & Florini, 1997; Singh *et al*., 1997).

The use of clean seeds, field sanitation, isolation from infected reservoirs, and the use of *Penisetum* windbreaks are suggested as cultural measures to control the disease (Allen, 1983). Foliar application of fungicides can also control the disease (Singh *et*

al., 1997). Mancozeb, captab, and biternol can effectively reduce the disease caused by *Alternaria cassiae* (van den Berg, 2000).

2.2.6 Brown blotch

Brown blotch is induced by two species, namely *Colletotrichum capsici* (H. Syd.) Butler and Bisby and *C. truncatum* (Schw.) Andrus and Moore (Singh *et al*., 1997). Symptoms range from seeds failing to germinate, seedling damping-off, stem or branch girdling, flowers aborting, immature pods mummifying, to ends of pods and leaves showing lesions (Emechebe & Florini, 1997; Singh *et al*., 1997). Primary sources of inoculum are infected seeds and infested debris and secondary sources are rain splash, wind and air currents (Singh *et al*., 1997). The same control as for anthracnose applies to brown blotch (Singh *et al*., 1997). Benomyl in combination with monocrotophos are effective in reducing brown blotch on cowpea (Olowe *et al*. 2003). According to Smith (1997), the disease can be effectively reduced by fungicides such as benomyl, captab, fludioxonil, and thiram. The pathogen can also be effectively controlled by application of *Trichoderma viridae* as a spore suspension foliar spray once or twice weekly from three days after inoculation (Bankole & Adebanjo, 1996).

2.2.7 Brown rust

Brown rust is caused by the fungus *Uromyces vignae* Barclay. Symptoms of brown rust are slightly raised brown or black pustules on the leaves (Allen, 1983; Singh & Rachie, 1985). Dispersal is through contact with people, animals, farm implements, wind and insects (Singh *et al*. 1997).

2.2.8 *Cercospora* and *Pseudocercospora* leaf spots

Cercospora leaf spot is induced by *Cercospora canescens* Ell. & Mart, while Pseudocercospora leaf spot is induced by *Pseudocercospora* (*Mycosphaerella*) *cruenta* (Sacc.) Deighton, formally *C. cruenta* (Emechebe & Shoyinka, 1985). The lesions are small, brown and circular with reddish-purple borders on leaves (Emechebe & Florini 1997; Boyhan *et al*., 1999). The pathogen overwinters on infected crop residues and infected seeds (Singh *et al*., 1997).

2.2.9 Powdery mildew

Cowpea is susceptible to powdery mildew during wet and humid conditions (Emechebe & Florini, 1997; Valenzuela & Smith, 2002). Powdery mildew is caused by *Oidium* spp., *Erysiphe polygoni* (DC) and *Sphaerotheca fuliginea* (Schelecht.) Pollacci. and it can be controlled by the use of resistant varieties and application of fungicides such as triadimefon (Singh *et al*., 1997). The symptoms are white, powdery growth consisting of oidia appearing on the upper surface of the leaf (Boyhan, 1999). Chlorotic and then brown patches also appear on the upper surface of the leaf, which finally result in the defoliation of the plant.

2.2.10 Seedling decay and damping-off complex

Seedling decay and damping-off occurs during pre-and post-emergence and they are induced by four pathogens which are: *Pythium aphanidermatum* [Edson] Fitzp, *Rhizoctonia solani*, *Colletotrichum capsici* and *Macrophomina phaseolina* (Tassi) Goid (Dorrance *et al*., 2001). Adandonon (2000) also found that damping-off in South Africa is mostly caused by *Pythium ultimum* Trow and *Rhizoctonia solani*. *Rhizoctonia solani* symptoms are characterized by reddish-brown lesions that are usually limited to the collar regions of the hypocotyls at which point the diseased seedling collapses (Agrios, 1997). *Pythium aphanidermatum* lesions move rapidly up the hypocotyls. They appear grey green and wet and eventually collapse. *Colletotrichum capsici* infected seeds fail to germinate and seedlings collapse. The symptoms are purplish-brown lesions that girdle the stem at soil level (Singh & Rachie, 1985). Aveling *et al*. (2001) found that damping-off and stem rot of cowpea disease can be hastened by high soil moisture. In their studies it was also confirmed that the infection rate is highest at the seedling stage.

2.3 *Rhizoctonia solani*

2.3.1 Introduction

Rhizoctonia solani is a soilborne pathogen with worldwide distribution and it is capable of attacking a tremendous range of host plants, causing seed decay, stem

canker, aerial leaf blight, storage rot and seedling damping-off in crops including carrot, soybean, potatoes and cowpea (Parmeter, 1970; Carisse *et al*., 2001; Harikrishnan & Yang, 2002; Thornton *et al*., 2004). *Rhizoctonia solani* is a complex and collective fungal species, which consists of strains that differ in host range, pathogenicity, cultural characteristics, and the way they respond to the environment (Jones *et al*., 1997; Dorrance *et al*., 2001).

Rhizoctonia solani is known as a basidiomycete fungus that does not produce asexual spores (conidia) and occasionally produces basidiospores (Ceresini, 1999). The mycelium of *R. solani* is colourless at first but becomes brown as the hyphae grow. *Rhizoctonia solani* is found in most agricultural soils and survives on plant residues and as microsclerotia (Laemmlen, 2004). Once *R. solani* is in the soil or seeds it moves quickly through the seedlings, killing those in its path (Carroll, 2004b). The symptoms start with seeds turning dark brown, then decaying until the brown, thread-like mycelium may be seen with a lens on the surface of the lesion or canker. Control of *R. solani* disease is difficult and it can be anticipated and prevented by using seed and transplant treatments before planting (Laemmlen, 2004).

Rhizoctonia solani infection and disease development can occur over a wide range of soil moisture levels. *Rhizoctonia solani* damage occurs at any time during the growing season; however, it is more severe on young seedlings (Dorrance *et al*., 2001). *Rhizoctonia solani* is capable of causing severe damage to beans often during the earlier stages of the growing season (Laemmlen, 2004).

2.3.2 Origin, taxonomy and distribution

Julius Kühn originally described the most widely recognised species of *Rhizoctonia* on potato in 1858 (Ceresini, 1999). *Rhizoctonia solani* belongs to the group “Mycelia sterilia” which does not produce asexual spores, but grows by producing thin vegetative hyphae (Carroll, 2004a). The sexual fruiting structures are basidiospores, which were first described in detail by Prillieux and Delacroiz in 1891 (Ceresini, 1999). This teleomorph is known as *Thanatephorus cucumeris* Dec. (Ceresini, 1999).

The mycelium consists of hyphae partitioned into individual cells by a septum containing a doughnut shaped pore. Since the fungi do not produce conidia, hyphal anastomosis criteria are used to place isolates of *Rhizoctonia* into taxonomically distinct groups called anastomosis groups (Laemmlen, 2004).

Rhizoctonia solani belongs to the Phylum Basidiomycota, class Hymenomycetes, Order Ceratobasidiales and Family Ceratobasidiaceae (Tsukiboshi *et al*., 2002). *Rhizoctonia solani* is characterised by cellular nuclear numbers greater than two close to the tips in young hyphae, wider main runner hyphae, buff-coloured to dark brown mycelium, and sclerotia irregular in shape, light to dark brown without differentiation (Ceresini, 1999). *Rhizoctonia solani* consists of similar morphological groups that share characteristics such as multinucleate cells with dolipores, production of sclerotia, and lack of conidia (Laroche *et al*., 1992).

Rhizoctonia solani is widespread in crop plants and cultivated land, but often also arises in uncultivated areas such as forests. Its distribution shows that the fungus has been present for a long time (Parmeter, 1970). It consists of many strains, distinguished on the basis of host range, virulence and type of attack on a specified host and its capability to grow and survive in the lower level of the soil, at the soil exterior, or as an aerial pathogen (Parmeter, 1970; Dorrance *et al*. 2003; Harikrishnan & Yang, 2004). The species evolved a strong association with other soil microorganisms in its ability to survive and grow through the soil effectively under a wide range of conditions (Parmeter, 1970; Ceresini 1999).

2.3.3 Ecology, epidemiology and environmental conditions

Rhizoctonia solani can be found in cool and warm soils, because the fungus causing the disease is active at different temperatures (Olsen & Young, 1998). Disease becomes severe when temperatures are adverse to the host (24-32ºC) (Jones *et al*., 1997). The mildly virulent strain causes most disease at 24ºC, while the moderately virulent strain causes disease at 32ºC and the highly virulent strain causes disease at 24, 27, and 32ºC (Parmeter, 1970; Harikrishnan & Yang, 2004). Rainfall followed by

cool weather in a subnormal rainfall season, high temperatures and soil moisture favour *R. solani* disease development (Parmeter, 1970; Dorrance *et al*., 2003).

The sources of inoculum for *R. solani* are natural soil, contaminated weed or rotation crops, plant debris and infected seeds (Parmeter, 1970). Survival and inoculum potential are influenced by soil factors such as temperature, moisture, pH, and competitive activity with associated organisms (Jones *et al*., 1997). According to Ceresini (1999), *R. solani* can survive for a long time by producing small, irregular shaped, brown to black structures (sclerotia) in soil and on plant tissue. It can also survive as mycelium by colonising soil organic matter as a saprophyte (Olsen & Young, 1998).

Rhizoctonia solani is an ectotrophic fungus that is well adapted to life outside the plant, for which the plant is merely a food source and infection of the plant allows the fungus to exploit these food sources (Keijer *et al*., 1997). Sclerotia and mycelium germinate by producing hyphae that attack food and fibre crops (Ceresini, 1999). The optimum temperature range for sclerotia production in *R. solani* is between 18 and 25 ºC (Harikrishnan & Yang, 2004). After attachment to the external surface of the host *Rhizoctonia* grows and causes disease. As the fungus continues to kill the plant cells the hyphae grow, colonising dead tissues after forming sclerotia (Agrios, 1997).

The pathogen invades the seed while still in the pod, decomposing the pod, or may infect from infested soil during planting. The problem of seed decay starts immediately after planting, before germination (Beker, 1947 and Neergard, 1958 as reported by Parameter, 1970). Damping-off might result from insufficient inoculum for fast infection to take place or from unfavourable conditions that do not favour the pathogen or the host (Agrios, 1997). Post-emergence damping-off occurs when the stem starts decaying at about soil level, causing it to fall because of lack of supporting tissues (Agrios, 1997). The pathogen sometimes attacks the roots of young plants causing root rot.

2.3.4 Symptoms

Rhizoctonia solani damage occurs at any time during the growing season, and the pathogen attacks mostly young seedlings (Dorrance *et al*., 2001). *Rhizoctonia solani* causes damping-off, with reddish-brown lesions, which appear on the stem at soil level and girdle the stem when conditions are favourable (Olsen & Young, 1998; Koster & Meer, 1990). The stem may sometimes be water soaked and soft, causing the plant to collapse (Olsen & Young, 1998). *Rhizoctonia solani* also causes seed rot, root rot, and lesions on hypocotyls (Dorrance *et al*., 2001).

Living infected seedlings have cankers, which are reddish-brown with lesions on the stem and roots (Ceresini, 1999). *Rhizoctonia solani* causes post-emergence damping-off known as wirestem on cole crops (*Brassica oleracea* L. var.), which is characterised by dark lesions of varying depth on the hypocotyls at or just above the soil level (Koster & Meer, 1990; Pullaro, 2003). When older plants are infected they become chlorotic, resembling plants with nitrogen deficiency (Dorrance *et al*., 2001). Cowpea pod that come in contact with the soil in warm wet areas develop brown rot often with alternating light and dark coloured concentric bands (Jones *et al*., 1997). Brown, coarse mycelium of the fungus appears on the surface of infected plant parts under moist conditions (Jones *et al*., 1997).

2.3.5 Control

Avoiding transmission by propagating materials and treatment of seeds with fungicides such as chloroneb can reduce disease incidence in the field and greenhouse (Olsen & Young, 1998). When preparing a seedbed avoid deep planting because it exposes more seedling tissue to infection and prolongs the period of susceptibility (Parmeter, 1970; Olsen & Young, 1998). In the greenhouse, chemical or heat pasteurised medium can be used to avoid disease occurrence (Jones *et al*., 1997). In field plantings, soil fumigation with a broad-spectrum fumigant controls the fungus (Jones *et al*., 1997). Mefanoxam with fludioxonil, or a combination of mefanoxam, fludioxonil, and azoxystrobin are effective in reducing *Rhizoctonia solani* root rot in

soyabean (Bucher & Pedersen, 2004). Tolclofos-methyl and flutolanil can be used to control *R. solani* if they are used in the same dosages (Koster & Meer, 1990). Mancozeb, copper oxychloride, carbendazim and metalaxyl were found to be effective in controlling dry corm disease caused by *R. solani* (Bhaskar *et al*., 2005b). According to Patricio *et al*. (2006), *R. solani* can be controlled by using solirisation in combination with fungicides such as pencycuron and procymidone on lettuce.

Biological control agents such as endophytic bacteria and fungal antagonists including *Trichoderma* spp. have shown potential for practical application in agriculture (Carisse *et al*., 2001; Parmeter, 1970). According to Krause *et al*. (2001), some of the biological control agents that can control *R. solani* are *Burkholderia* spp., *Pseudomonas* spp., *Bacillus* spp. and *Somyctreptes* spp. (Krause *et al*. 2001). The bacterium *Paenibacillus illinoisensis* KJA-424 also suppresses the symptoms of damping-off in seedlings caused by *R. solani* (Jung *et al*., 2003). Combinations of *B. subtilis* RB14-C with *B. cepacia* B.Y. can reduce damping-off caused by *R. solani* (Szczech & Shoda, 2004). The fungus *Cladorrhinum foecundissimum* Saccardo and Marchal. can be used to reduce the incidence of *R. solani* on eggplant and pepper (Lewis & Larkin, 1998).

Crop rotation can be used as an effective control measure for the disease (Parmeter, 1970; Dorrance *et al*., 2001). Use of mulches and preventing direct contact of the plant with the soil under warm, moist conditions can control the disease effectively (Jones *et al*., 1997). Avoiding excessive application of fertilizer (Dorrance *et al*., 2003), good growing conditions and preventing plant injury, especially by nematodes, reduces root and foot rot caused by *R. solani* (Jones *et al*., 1997). Careful irrigation of the seedbed, and planting when environmental conditions are favourable for rapid growth of seedlings can reduce high soil moisture (Olsen & Young, 1998). Use of herbicide-tolerant cultivars could reduce herbicide-related stresses and decrease diseases compared with conventional cultivars, which lack herbicide tolerance (Harikrishnan & Yang, 2002).

2.4 *Pythium ultimum*

2.4.1 Introduction

The disease caused by *Pythium ultimum* var *ultimum* Trow. is characterised by seedling damping-off, smaller and deformed primary true leaves, plant stunting, reduced tillering, loss of fine feeder roots, and poor yield (Paulitz & Adams, 2003). The genus *Pythium* is ecologically and physiologically dispersed worldwide. *Pythium* spp. are rapidly growing fungi that need minimal nutrition for growth of the hyphae (Carroll, 2004b).

Effects of root-infecting soil-borne microorganisms vary from causing death to more subtle effects on the growth of roots (Mihail *et al*., 2002). Species of *Pythium* such as *P. dissotocum* Drechs., *P. acanthicum* Drechsler., *P. torulosum* Coker and Patterson. and *P. rostratum* E.J. Butler reduce root system length whereas others like *P. ultimum, P. irregulare* Buisman and *P. sylvaticum* Campbell and Hendrix can cause pre- and post-emergence damping-off (Mihail *et al*., 2002; Paulitz & Adams, 2003).

2.4.2 Origin, taxonomy and distribution

According to Paul (2001), members of the species *Pythium* are common and distributed worldwide. More than 200 species of this genus are described of which 130 are recognized (Paul, 2004). The genus *Pythium* contains species ranging from saprophytic facultative parasites with extensive host ranges to highly pathogenic species with limited host ranges (Chen *et al*., 1992).

According to Abdelghani *et al*. (2004), the members of the genus *Pythium* belong to the class Oomycetes and they are known and represented worldwide. *Pythium* spp. are found in soil, sand, pond and stream water and their sediments (Moorman, 2004). All species of *Pythium* produce white, cottony, coenocytic mycelium and reproduce asexually by producing sporangia of different sizes depending on the species (Jones *et al*., 1997). *Pythium* grows slowly with large globose and lemoniform, terminal, intercalary to catenulate sporangia, smooth walled oogonia and hypogynous

antheridia (Paul, 2004). According to Green & Jansen (2000), oospores of *Pythium* are thick walled, resistant to desiccation and can survive for long periods in dry soil. Oospores and sporangia are the primary survival structures and sources of inoculum for most *Pythium* species. They may also overcome fungistasis by germinating directly through a germ-tube.

The taxonomy of *Pythium* is based on comparison of morphological characteristics and temperature-growth relationships of different members of the genus (Paul, 2001). However, *Pythium* spp. are sometimes difficult to identify using morphological characteristics and most species are asexual or heterothallic and do not form sexual structures in culture (Paulitz & Adams, 2003). Comparative studies of the internal transcribed spacer (ITS) regions of ribosomal (rRNA) genes are important in fungal taxonomy to distinguish various species within a genus (Paul, 2004).

Oomycetes belonging to the genus *Pythium*, are not considered as true fungi (Paulitz, 1991; Abdelghani *et al*., 2004). Members of the genus *Pythium* are filamentous, heterotrophic microorganisms, which share some ecological and morphological similarities with the true fungi (Mihail *et al*., 2002). The hyphae grow both inter-and intracellularly. When invading the plant tissue they penetrate through plant cells, and pectic enzymes dissolve the middle lamella of the cell wall and soften the tissues (Carlille & Watkinson, 1996).

2.4.3 Ecology , epidemiology and environmental conditions

Most *Pythium* spp. are facultative saprophytes as well as pathogenic on plants, and they cause important losses of economic crops on a worldwide basis. The behaviour of *Pythium* in soil is moderated by environmental factors such as moisture, soil pH, and the presence of soil minerals (Martin & Loper, 1999). Soil moisture influences the mobility of zoospores which require free water, and the type of reproductive spores formed by *Pythium* spp.. Temperatures that favour infection vary according to the type of species e.g. Green & Jansen (2000) observed less pre-emergence damping-off of sugar beet and watermelon caused by *P. ultimum* at 30 to 35^{o}C, whereas *P. irregulare* causes damping-off at 5^{o}C only. Maximum infection is observed at pH

levels between 4.8 and 6.9, with a decrease in infection at pH 7.6. The effect of pH on *Pythium* varies among and within species (Green & Jansen, 2000).

According to Green & Jansen (2000), *P. ultimum* attacks young and succulent host tissue, infecting seeds and radicals resulting in seed rot and pre-emergence damping-off. It can also infect newly emerged seedlings at ground level, resulting in post-emergence damping-off (Adandonon *et al*., 2003; Moorman, 2004). Secondary wall thickening occurs in the cells of stems and main roots and after its occurrence, infection is restricted to the root tips or feeder roots, limiting plant vigour and yield which can result in plant death (Olsen & Young, 1998). *Pythium* spp. are commonly found where soil moisture and plant density are high, usually in greenhouses, horticultural and forest tree nurseries (Green & Jansen, 2000). There is a connection among ecological factors and *Pythium* spp., according to Paulitz & Adams (2003), who found that *P. ultimum* and *P. irregulare* were frequently collectively and absolutely linked with average rainfall. In contrast, *P. sylvatium* was negatively correlated with rainfall and appeared to be related to higher temperatures and drier conditions (Paulitz & Adams, 2003).

When environmental conditions are favourable, particularly where water logging is high, sporangia or oospores may produce zoospores (Olsen & Young, 1998). Zoospores are mobile in water because they are biflagellate whereas oospores and sporangia have limited mobility. After primary inoculum infection some *Pythium* spp. produce secondary inoculum. Zoospore dissemination occurs through soil or growth media. The degree of dissemination is limited depending on the water potential, soil texture and growth medium (Green & Jansen, 2000). For those species that do not produce zoospores, the sexual structures are often referred to as conidia or hyphal swellings, while those that do release zoospores are referred to as zoosporangia. The mycelial growth from infected plant tissue is important as a means of spread of damping-off and root rot (Martin & Loper, 1999).

2.4.4 Symptoms

According to Olsen & Young (1998), infected seeds fail to germinate, become soft and mushy and later turn brown, shrink, and finally disintegrate. *Pythium ultimum* causes post-and pre-emergence damping-off and can also reduce growth in mature plants through chronic root tip attack (Yuen *et al*., 1993; Aveling & Adandonon, 2000). Young seedlings can be attacked before emergence and infection can spread quickly. Invaded cells collapse, and seedlings are infested by the oomycetes and die (Olsen & Young, 1998).

Emerged seedlings are attacked at the roots or anywhere below the soil line. Infected parts are water-soaked and discoloured, and they soon collapse (Aveling & Adandonon, 2000). The basal part of the stem turns soft and becomes thinner than the upper parts as the fungus grows. The fungus continues to infect the fallen seedlings, which wither and die (post-emergence damping-off). According to Chen *et al*. (1992), *Pythium* species can cause feeder root necrosis, cryptic disease, subclinical infection and replant diseases in many crops.

2.4.5 Control

Pythium ultimum in the greenhouse can be controlled by soil steam sterilization, dry heat or use of chemically treated seeds (Jones *et al*., 1997). Application of metalaxyl as a seed treatment plus *Trichoderma virens* J.H. Miller, J.E. Giddens and A.A. Foster, is effective in controlling seedling diseases caused by *P*. *ultimum* (Howell, 1991; Howell *et al*., 1997).

Soil fumigants should be applied before sowing, especially a methyl bromide-chloropicrin combination (Cordel *et al*., 2002). Use of soil fungicides such as propomocarb hydrochloride, etridiazole, chloroneb or metalaxyl as a drench are also recommended (King & Parke, 1993; Cordel *et al*., 2002). Mefenoxam is effective in reducing the disease caused by *Pythium ultimum* on potatoes as reported by Taylor *et*

al. (2004). Ghate *et al*. (1991) found that metalaxyl in combination with flutolanil is effective in reducing the disease caused by *Pythium* spp. on cucumber.

According to Paulitz (1991), treatment of germinating seeds with fluorescent *Pseudomonas* controls *Pythium* damping-off. Use of saprophytic Ascomycetes, such as *Chaetomium globosum* Kunze: fr., which are effective against most soilborne and seedborne diseases, control *Pythium* (Di-Pietro *et al*., 1992). It was found that *Pythium oligandrum* Drechs. and *P. nunn* Lifshitz. reduce diseases such as damping-off of tomato caused by *P. ultimum* and *P. aphanidermatum*, when applied to seedlings prior to transplanting (Martin & Loper, 1999).

Pseudomonas putida, which is a biological control agent of *Fusarium* wilt of cucumber, can also control damping-off caused by *Pythium* spp. (Paulitz, 1991). *Bacillus* spp. L324-92 has also found to suppress *Pythium* rot of wheat caused by *P. irregulare*, *P. ultimum*, take-all disease and *Rhizoctonia* root rot (Martin & Loper, 1999). According to Lumsden & Locke (1989), *Gliocladium virens* is an antagonistic fungus that can control damping-off caused by the fungi *Pythium ultimum* and *Rhizoctonia solani*.

Crop rotation with a non-susceptible host and improved drainage can effectively control the disease in the field (Cordel *et al*., 2002). Good soil drainage and good air circulation, planting during favourable temperatures, avoidance of excessive amounts of nitrates can reduce disease incidence of *P. ultimum* (Agrios, 1997; Jones *et al*., 1997).

2.5 *Fusarium solani*

2.5.1 Introduction

Fusarium solani f.sp. *phaseoli* is an asexual, soilborne fungus found in agricultural soils worldwide. It is a diverse soil saprophyte and facultative parasite associated with wounds and other infections that cause root rot, stem cankers and storage rots of many plants (Marasas *et al*., 1984). Of all the diseases caused by the pathogen *F. solani*,

root rot is regarded as a serious disease in most bean producing countries worldwide (Nelson *et al*., 1981).

Fusarium solani is commonly found on plants and in mycoflora of commodities such as rice, bean, and soybean (Fry, 2004). *Fusarium solani* causes a variety of diseases on different hosts and can attack several plants including most greenhouse vegetables (Nishijima, 1993).

2.5.2 Origin, taxonomy and distribution

Fusarium species belong to the Kingdom: Fungi, Phylum: Deuteromycota, Order: Hypocreales, Family: Hypocreaceae and the Genus: *Fusarium* (Fry, 2004). *Fusarium solani* was described and clearly illustrated as *Fusisporium solani* and transferred to *Fusarium* as reported by Booth (1971).

The *Fusaria* are widely distributed in soil, on subterranean and aerial plant parts, and debris. The population of *Fusarium* spp. in agricultural fields is often as high as 100,000 propagules per gram of soil or more (Nelson *et al*., 1981). Humans also contribute to the dissemination of *Fusarium* pathogens through the distribution of infected or infested seeds or other plant material (McGovern *et al*., 2001). The fungi are commonly found in tropical and temperate regions and exist as common soilborne fungi, which include both saprophytes and parasites (McGovern *et al*., 2001). Survival of *F. solani* in agricultural land is enhanced by temporary supplies of nutrients in diffusates from non-susceptible plants and crop residues, which enable the fungus to vegetate and form new chlamydospores (Nelson *et al*., 1981). Some parasitic members are important root and crown rot pathogens, while others have been implicated as canker-causing organisms of several hardwood trees (Nelson *et al*., 1981).

All *Fusarium* species have one taxonomic feature in common: A production of distinctly foot shaped macroconidia. *Fusarium solani* f.sp. *radicicola* has the “coeruleum” look (conidia are short with obtuse extremities) whereas in *F. solani* f.sp. *phaseoli*, conidia are long and pointed, and the saprophytic forms are

intermediate (Nelson *et al*., 1981). The spores of *F. solani* are disseminated by water-splash, pruning knives and other tools such as, clothing or worker's hands (Cercauskas, 2001) and the pathogen overwinters as chlamydospores in naturally infested soil (Davis *et al.*, 2004).

2.5.3 Ecology, epidemiology and environmental conditions

All species of *Fusarium* can be found in noncultivated land with *Fusarium* o*xysporum, F. solani* and *F. roseum* being the most widespread and predominant species. *Fusarium* species are associated with roots and organic matter such as debris, and they normally occur in all climates (Nelson *et al*., 1981).

The highest numbers of *Fusarium* species are found in the upper horizon, usually the top 5-15 cm of soil (Nelson *et al*., 1981). This zone represents the portion of the soil profile most affected by farming operations such as tillage, fertilization, liming, herbicide application and irrigation (Rupe *et al*., 1999). The upper 15 cm zone is also associated with greater amounts of biological activity (Toussoun & Nelson, 1976).

The optimum temperature for growth of *F. solani* is 27-31 °C, optimum pH is 7.8 and humidity should be at a maximum (Glen *et al*., 2003). According to Saremi *et al*. (1999), colonisation of roots by *F. solani* is severe at temperatures ranging from 25-30 °C. The spores that are released during the night, cause a more rapid rate of disease development because high relative humidity and dew occur during the night (Cerkauskas, 2001). Initial infection occurs mostly during cool, wet weather in the growing season. Poorly drained soil promotes disease development (McGovern *et al*., 2001).

Fusarium solani survives as chlamydospores which are terminal, single, catenulate or intercalary. Chlamydospores germinate when near germinating bean seeds or root tips and they can also reproduce in soil near seeds of many non-susceptible plants and other organic matter (Nelson *et al*.,1981). The fungus penetrates the plant directly or

through stomata or wounds where infection hyphae multiply in the intercellular spaces of the cortex until they are stopped by the endodermis (Agrios, 1997).

Conidia are produced in large quantities during the imperfect stage when conditions are favourable and they are dispersed by water splash and other factors. The ascospores germinate during periods of high humidity or may survive for a long period until the conditions become favourable for infection (Davis *et al*., 2004). When conditions are unfavourable for optimum root growth *Fusarium* reduces root volume and efficiency and the primary roots are killed (Nelson *et al*., 1981)

2.5.4 Symptoms

According to Koenning (2004), *F. solani* causes seedling diseases by attacking seeds before germination or attacking young seedlings before or after emergence. The first symptoms are reddish streaks on the hypocotyls and tap root, which appear a week after plant emergence (Nelson *et al*., 1981). The reddish discolouration increases and coalesces to cover the entire belowground stem and root system, giving it a brown, corky appearance (Nelson *et al*., 1981). The red colour turns brown with age and longitudinal fissures develop in the cortical tissue of the affected areas. As the infection becomes severe, the entire root system may be attacked and destroyed.

Soft, dark brown or black cankers develop on the stem nodes and may girdle the stem during disease development (Cerkauskas, 2001). Foliar symptoms develop shortly after the onset of crop flowering and the most characteristic symptoms occur on the leaves (Mueller *et al*., 2002). These include mottling, mosaic, interveinal chlorosis and necrosis on the upper leaves, defoliation and premature plant death. Other symptoms include root rot, crown rot, vascular discolouration of stems and pod abortion (Fry, 2004). The disease causes more damage to stressed plants under conditions of reduced root growth caused by drought, poor nutrition, or oxygen stress caused by wet soil (Davis *et al*., 2004).

Lesions are small, depressed and often covered by a combination of mycelia and conidial masses (Nishijima, 1993). Light orange coloured structures may develop on the lesions, which are the fruiting bodies of the fungus (Cerkauskas, 2001).

2.5.5 Control

General disinfections, which include soil and greenhouse fumigation, can effectively control the fungus (Cerkauskas, 2001). *Fusarium* is typically found on diseased seedlings, therefore seed-applied fungicides are effective in controlling *Fusarium* (Koenning, 2004). Treating seed with fungicides also helps in controlling decay and other diseases caused by seed-borne pathogens (Loria, 1993). Thiabendazole and thiophanate methyl are used to prevent seed decay caused by *Fusarium solani*. Essential oils such as oregano, thyme and dictamnus can be used to inhibit the growth of *Fusarium* spp. (Daferera *et al*., 2002). Bhaskar *et al*. (2005a) reported the effectivity of mancozeb, copper oxychloride, carbendazim and metalaxyl in reducing dry corm rot disease caused by *F. solani* in colocasia. According to Allen *et al*. (2004), mancozeb and hydrogen dioxide are effective in reducing the disease caused by *F. solani* on long leaf pine.

Biological control of *Fusarium* root and stem rots can be achieved by incorporating organic materials such as barley stow and chitin in the soil, to favour the increase of fungi and bacteria antagonistic to *Fusarium*, or by treating seeds or transplants with spores of fungal antagonists or antagonistic bacteria (Agrios, 1997). Integration of *Bacillus-Rhizobium* inoculants and tillage can be used to control bean and soybean root rot caused by *F. solani* (De Jensen *et al*., 2004). *Gliocladium roseum* Syn. (ACM941) is effective in controlling *F. solani* (Xue, 2003). The results obtained by Oyarzun *et al*. (1994) suggested that some strains of *F. oxysporum* are capable of reducing the disease incidence caused by *F. solani*. In another experiment conducted by Selvarajan & Jeyarajan (1996), it was found that some strains of *B. subtilis*, *Pseudomonas fluorescence* and *Trichoderma* spp. can effectively reduce the sporulation of *F. solani*.

According to Cerkauskas (2001), good sanitation practices and maintaining good ventilation and drainage to avoid high relative humidity, which favours germination of ascospores, controls the fungus. Cleaning and disinfecting of the seed storage area before preserving seed can help in prevention of *F. solani* infection (Loria, 1993). Soil sterilization, use of healthy propagative materials, and rotation with non-susceptible crops like maize can control the disease, by reducing *F. solani* inoculum (Agrios, 1997; Davis *et al*., 2004).

Fusarium solani can also be controlled by avoiding excessive fertilization that can contribute to salt damage and by provision of favourable growing conditions by avoiding stress caused by excess water, prolonged drought and soil compaction (Davis *et al*., 2004). According to Bourbos *et al.* (1997), a combination of calcium cyanamide and soil sterilization reduces the infection caused by *Fusarium solani*.

2.6 Conclusion

In most parts of the savannah-zone of sub-Saharan Africa where provision of food and livestock are serious problems for poor farmers, cowpea fills a significant gap in the farming system (Singh & Eaglesfield, 2000) as it is a valuable protein source, which contributes in overcoming the protein deficit in southern Africa (Umapathy *et al*., 1998). There is a great demand for cowpea for human consumption in west and central Africa (Langyintuo *et al*., 2003). There are great prospects for adoption of cowpea to alleviate poverty and malnutrition and to contribute to the sustainability of African agriculture systems through profitable crop and livestock integration (Inaizumi *et al*., 1999).

The major constraints to cowpea production in Africa are pests and pathogens, which can sometimes lead to low yields or total yield loss (Alabi *et al*., 2003). Poor farmers are unable to control these pests and pathogens because of financial constraints, so there is a need for production of alternative control measures that could be affordable.

The pathogen *Pythium ultimum* lives as a saprophyte occupying a wide range of terrestrial and aquatic habitats (Abdelghani *et al*., 2004). The fungus causes serious

losses in field crops, nurseries and in ornamental plants (Laemmlen, 2004). Effective control can be achieved by preventing the fungus from invading the field area or by using an integrated pest control strategy (Paul, 2001).

Rhizoctonia solani occurs in both light, well drained and in heavy, poorly drained soil (Dorrance *et al*., 2001). Disease caused by *R. solani* is difficult to control, therefore it should be prevented before planting (Laemmlen, 2004). Control of the fungus should start during land preparation until storage, thus for effective control a method of integrated pest control should be implemented (McGovern *et al*., 2001).

Fusarium solani causes common, persistent, and damaging diseases in most field crops and major potted and field grown ornamentals (McGovern, 2001). For effective disease management there is a need for knowledge of pathogen survival, spread (including insects and other vectors) and conditions favouring infection (Fry, 2004). *Fusarium solani* like other soilborne diseases, causes serious losses to cowpea. Although chemical control is effective against *F. solani*, there is a need for development of other alternative control measures that are affordable for poor farmers.

Rhizoctonia solani, P. ultimum and *F. solani* cause great loss in cowpea yields and they are considered as future threats to cowpea production. There is a need for research on these diseases as they threaten cowpea production industries worldwide.

CHAPTER 3

MATERIALS AND METHODS

3.1 Fungi

Three pathogens viz. *Rhizoctonia solani* (UPGH122), *Fusarium solani* (UPGH112), and *Pythium ultimum* (UPGH 050) were obtained from the fungal culture collection in the Department of Microbiology and Plant Pathology at the University of Pretoria.

The pathogens were sub-cultured by placing a mycelial disc (4 mm) of each fungus on potato dextrose agar (PDA) (Merck, Johannesburg) in the centre of 90 mm Petri dishes. The cultures were incubated under fluorescent light at 25 ^{o}C for seven days before use.

3.2 Biological control agent

A bacterial culture (B5B - *Bacillus cereus*1) was obtained from the Department of Microbiology and Plant Pathology. The bacterium was sub-cultured on nutrient agar (Merck, Johannesburg) by means of streaking and incubated under fluorescent light at 25 ^{o}C for two days. For the *in vivo* experiments, the bacterial cells were harvested and 1 ml of the bacterial solution was subsequently dispensed into the prepared 500 ml nutrient broth. The nutrient broth was then put on a rotary shaker for 48 hr at 1000 rpm at room temperature. Ringers solution was prepared by mixing 1 L of water with two Ringers tablets. From the prepared Ringers solution, 250 ml was poured into an Erlenmeyer flask and of the remaining Ringers solution, 9 ml was poured into each of the ten sterilised test tubes.

Each of eight sterilised centrifuge tubes was half-filled with inoculated broth and centrifuged at 3000 x g for 20 minutes in a Sorval Super Speed centrifuge with a GSA rotor. After centrifuging the supernatant was discarded and each tube was filled halfway with the prepared Ringers solution. The tubes were then vortexed until all pellets/residue (bacteria) were dissolved. The solution was placed in a 250 ml

Erlenmeyer flask before preparing a dilution series using the ten test tubes containing Ringers solution. One millilitre of the 10^{-3} dilution was dispensed onto a Petroff-Hauser counting chamber and ten blocks at a time were counted three times and the average was determined.

3.3 Fungicides

The following fungicides were supplied by Syngénta South Africa (Pty) Ltd: Apron®XL (metalaxyl 350 gai/L), Celest® (fludioxonil 240 gai/L) and Subdue®MAXX (mefenoxam 240 gai/L).

3.4 *In vitro* culture essay

Potato dextrose agar was augmented with the various fungicides at the an amount of: Apron® 0.21 ml/L medium, Celest® 0.25 ml/L medium and Subdue® 0.27 ml/L medium. The media was then poured into Petri dishes (90 mm) and allowed to solidify.

A mycelial disc (5 mm diameter) of a seven-day-old PDA culture of each of the three fungi *Rhizoctonia solani*, *Fusarium solani* and *Pythium ultimum* was placed in the centre of each Petri dish. To test the inhibitory activity of the bacterium B5B (*Bacillus cereus*1), the bacterium was streaked on unamended PDA on opposite sides of the mycelial disc using a loop. There were three replicates of twelve Petri dishes per treatment for each pathogen. The Petri dishes were sealed with parafilm and incubated under fluorescent light at 25 ºC for nine days. The diameter of mycelial growth per Petri dish was recorded in millimetres on the third, sixth and the ninth day after inoculation. The experiment was repeated three times.

3.5 *In vivo* greenhouse trials

Polystyrene seedling trays with 128 cells were filled with steam pasteurised growing medium (Braaks Lawn Dressing) bought from a nursery in Pretoria. The growing medium was drenched with tap water one day before pathogen inoculation. Fungal

cultures were prepared as described in 3.1 and two mycelial discs (4 mm diameter) were then placed at a depth of 3 cm, in each cell of seedling trays a day before planting.

Cowpea seeds (cv. Pietersburg Blue) were obtained from the Dry Bean Seed Producer's Organisation, Pretoria. Seeds were planted by hand 3 cm deep in seedling trays (56 seed/tray). After sowing, the *B. cereus*1 isolate B5B, prepared as described in 3.3, was applied to the growing medium at an amount of 3 ml/cell using a bacterial cell suspension containing 10^6 cell/ml. Initially fungicides were applied to the growing medium as drench treatments after sowing at the recommended concentrations: Apron® at 0.53 ml/1.5 L water/m^2, Celest® at 0.67 ml/1.5 L water/m^2 and Subdue® at 0.77 ml/1.5 L water/m^2. Subsequently, fungicides were applied twice before harvesting using a hand-held sprayer (Knapsack sprayer) on the 14th and 28th days after planting. On the day that the plants received chemical treatment, they were not watered to avoid leaching of the chemicals.

Seedling trays were placed randomly in a greenhouse with four replications per pathogen per treatment, each replicate consisting of 56 plants. Plants were maintained at temperatures between 22-25 ºC and watered daily with tap water until harvesting. The experiment was repeated three times.

Percentage emergence was recorded at harvest on the 35th day after planting. Emerged seedlings were counted per replicate, per pathogen per treatment and the average was calculated. Percentage diseased plants showing symptoms were recorded once seedlings had been removed from the growing medium. Shoot lengths were measured from the soil level a day before harvesting and the averages calculated.

After harvesting, roots were washed with tap water and disease symptoms on the roots and stems were recorded. Roots were then excised from the shoots with scissors, placed into brown paper bags (28 x 15 cm) dried for 48 hrs in a Fixed Featured drying oven (custom made) at 65 ^{o}C at the Department of Botany, University of Pretoria. The dry mass of both roots and shoots was recorded.

3.6 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on the data of *in vitro* and *in vivo* experiments and means were separated using the Student t-test ($P = 0.05$).

CHAPTER 4

RESULTS

4.1 *In vitro* study

4.1.1 *Rhizoctonia solani*

The biological control treatment (*B. cereus*1) significantly reduced mycelial growth on the third and the ninth day when compared to the untreated control (Table 4.1). However, the same results were not obtained on the sixth day. All three fungicides significantly reduced the mycelial growth of *R. solani* on the third and ninth day (Table 4.1). However, only Celest® was able to significantly reduce mycelial growth on the sixth day.

4.1.2 *Pythium ultimum*

The results indicated that *B. cereus*1 was unable to reduce mycelial growth of the pathogen throughout the experiment when compared to the untreated control (Table 4.1). With the exception of Celest® on the ninth day, all fungicides significantly reduced mycelial growth (Table 4.1).

4.1.3 *Fusarium solani*

*Bacillus cereus*1 treatment significantly reduced mycelial growth of the pathogen from the third to the ninth day when compared to the control (Table 4.1). All fungicide treatments significantly reduced mycelial growth of the pathogen from the third to the ninth day when compared to the untreated control (Table 4.1).

Table 4.1 Effect of fungicides and *Bacillus cereus*1 on *in vitro* mycelial growth of *Rhizoctonia solani, Fusarium solani* and *Pythium ultimum*

Treatments	Colony diameter (mm)			Inhibition (%)		
*B. cereus*1	Third Day	Sixth Day	Ninth Day	Third Day	Sixth Day	Ninth Day
R-control	2.57b*	5.52a	8.10b			
R+B	2.40a	5.10a	6.02a	6.62	7.61	25.68
P-control	8.47a	8.50a	8.47a			
P+B	8.40a	8.50a	8.47a	0.83	0.00	0.00
F-control	1.90b	4.89b	6.95b			
F+B	1.58a	3.63a	4.77a	16.86	25.77	31.37
Fungicides						
R-control	2.18c	5.67b	8.30c			
R+Apron®	1.47b	5.50b	6.63b	32.57	2.99	20.12
R+Subdue®	1.23b	5.77b	7.17b	43.58	1.76	13.61
R+Celest®	0.80a	2.67a	4.17a	63.30	52.91	49.76
P-control	6.67c	8.48c	8.47b			
P+ Apron®	0.00a	0.00a	0.00a	100	100	100
P+ Subdue®	0.00a	0.00a	0.00a	100	100	100
P+ Celest®	1.30b	6.30b	8.37b	80.51	25.71	1.18
F-control	2.17b	4.97c	6.90c			
F+ Apron®	0.83a	2.40b	3.30b	61.75	51.71	52.17
F+ Subdue®	0.60a	1.30a	2.10a	72.35	73.84	69.56
F+ Celest®	0.53a	2.27b	2.40a	75.38	54.33	65.22

F=*Fusarium solani*, R=*Rhizoctonia solani*, P=*Pythium ultimum* and B=*Bacillus cereus*1. *Values in a column per pathogen per treatment followed by the same letter are not significantly different (*P=0.05*).

4.2 *In vivo* greenhouse trials

4.2.1 *Rhizoctonia solani*

All the pathogen-inoculated treatments showed reduced seedling emergence when compared to the uninoculated control except in the second trial (Table 4.2). However, all the treatments, with the exception of Subdue® in the first trial significantly increased seedling emergence when compared to the inoculated control. Although all the treatments, except Apron® in the first trial significantly reduced the percentage diseased plants when compared with the inoculated control, they did not completely control *R. solani* when compared to the uninoculated control.

All the treatments in all trials reduced plant height, resulting in stunting, with the exception of the second trial where only Celest® reduced plant height when compared to the uninoculated control (Table 4.2). Likewise, all treatments reduced dry shoot and root mass when compared to the uninoculated control, except for the *B. cereus*1 in the second trial and Celest® in the first trial.

Mixed results were obtained among the three trials when comparing plant height and dry shoot and root mass of the treatment with the inoculated control (Table 4.2). The biological control agent (*B. cereus*1) significantly increased plant height in all the trials when compared to the inoculated control. The same trend was observed in terms of dry shoot mass except in the first trial, where it was significantly lower.

Although the Celest® treatment significantly increased plant height in trial one and two when compared to the inoculated control, similar results were not obtained in the third trial. However, dry shoot mass was significantly higher in all the trials when treatments were compared to the inoculated controls. Only the dry mass of roots in the second trial was significantly higher than the inoculated control (Table 4.2).

In the first trial the Subdue® treatment did not differ significantly from the inoculated control. This was however not the case in the other two trials with the exception of dry root mass in the third trial (Table 4.2).

The Apron® treatment significantly increased plant height and dry shoot and root mass in all the trials when compared to the inoculated control except for the third trial where the dry shoot and root mass did not differ from the inoculated control (Table 4.2).

4.2.1.1 Symptoms

During harvesting it was observed that seeds that failed to germinate, were brown, and water-soaked. *Rhizoctonia solani* caused root rot and reddish-brown sunken lesions on the stem below and above the soil line (Figure 4.1).

Figure 4.1 Disease symptoms caused by *Rhizoctonia solani* on cowpea seedlings (a); and non-infected seedlings (b).

Table 4.2 Effect of *Bacillus cereus*1 and fungicides on infection, emergence and plant height of cowpea in *Rhizoctonia solani* inoculated soil

Treatments	Emergence (%)	Diseased seedlings (%)	Plant Height (cm)	Dry Shoot Mass (g)	Dry Root Mass (g)
Trial 1					
Inoculated control	21.50a*	66.95c	4.887a	5.275a	1.125a
Uninoculated control	64.00c	0.00a	11.75c	12.91c	4.93c
B5B**	39.00b	53.75b	7.53b	6.73ab	2.93b
Apron®	41.75b	62.08cb	7.60b	8.175b	3.38b
Subdue®	30.00ab	54.98b	5.57a	6.55ab	1.40a
Celest®	39.50b	54.17b	7.70b	12.91c	4.93c
Trial 2					
Inoculated control	49.25a	46.50c	8.25a	7.55a	3.05a
Uninoculated control	81.75b	0.00a	17.0c	19.80d	9.18c
B5B	73.00b	20.00b	14.75cb	17.50cd	5.89a
Apron®	79.25b	8.75b	14.50cb	13.75b	5.75b
Subdue®	79.75b	25.00b	12.25cb	14.40cb	6.25b
Celest®	73.00b	17.75b	12.75b	14.10b	5.88b
Trial 3					
Inoculated control	33.0a	79.72c	4.075a	3.865a	0.575a
Uninoculated control	78.75d	0.0a	11.48d	14.44d	2.727b
B5B	51.50c	34.38b	9.25c	8.760c	1.340a
Apron®	42.75b	31.25b	6.025b	4.165a	0.49a
Subdue®	42.0b	39.58b	6.10b	6.405b	0.57a
Celest®	40.75b	40.80b	4.5a	6.89cb	1.467a

**B5B=*Bacillus cereus*1. *Value is a mean of three replicates of 12 Petri dishes. Value is a mean of four replications of 56 seedlings. Values in a column per trial followed by the same letter are not significantly different ($P = 0.05$).

4.2.2 *Pythium ultimum*

All treatments in all trials caused increased seedling emergence, except Celest® and Subdue® in the third trial as compared to the inoculated control treatment (Table 4.3). In the second trial all treatments increased emergence to the level of the uninoculated control. However, in the third trial only the biological control agent (*B. cereus*1) managed to increase emergence to the level of the uninoculated control.

The disease was significantly reduced by all treatments in all trials when compared to the inoculated control. However, only the *B. cereus*1 treatments in the third trial were comparable with the uninoculated control (Table 4.3).

All treatments significantly increased plant height, and dry shoot and root mass in trial one and two when compared to the inoculated control treatment. However, they did not differ significantly in height from the uninoculated control in trial two. Only the biological control treatment (*B. cereus*1) in trial three significantly increased plant height, dry shoot and root mass in comparison to the uninoculated control, whereas in trial one all treatments had similar dry root masses, except for Subdue® (Table 4.3).

4.2.2.1 Symptoms

During harvesting it was noticed that some seeds had failed to germinate and they were brown and water-soaked, whereas some seedlings showed symptoms of root rot and stunting. The basal part of the stem was soft and reduced in diameter when compared to the upper part of the stem (Fig 4.2).

Figure 4.2 Disease symptoms caused by *Pythium ultimum* on cowpea seedlings (a); and non-infected seedlings (b).

Treatments	Emergence (%)	Diseased seedling (%)	Plant Height (cm)	Dry Shoot Mass (g)	Dry Root Mass (g)
Trial 1					
Inoculated control	41.10a*	60.26c	3.60a	3.60a	1.43a
Uninoculated control	83.50d	0.00a	12.08d	19.5d	3.96d
B5B**	62.95cb	12.06b	8.66cb	12.84bc	3.50cd
Apron®	62.68cb	14.73b	9.82c	12.64bc	3.55cd
Subdue®	56.00b	13.85b	7.14b	9.41b	2.67b
Celest®	71.00c	10.72b	9.65c	13.83c	3.6cd
Trial 2					
Inoculated control	52.00a	46.83c	9.50a	5.68a	1.45a
Uninoculated control	86.25b	0.00a	16.50b	16.83d	7.97d
B5B	81.25b	16.75b	13.75b	10.83cb	4.41c
Apron®	75.00b	19.00b	15.25b	12.82c	4.04c
Subdue®	82.25b	16.75b	13.75b	10.83cb	4.41c
Celest®	78.75b	16.75b	13.70b	9.63b	2.73b
Trial 3					
Inoculated control	42.25a	68.30d	4.80a	7.865a	0.68a
Uninoculated control	77.25c	0.00a	10.70d	14.39b	3.02bc
B5B	69.75c	9.38ab	9.98cd	14.68b	3.70c
Apron®	53.00b	27.07c	8.70cb	8.85a	1.30ab
Subdue®	50.25ab	22.92c	8.15b	6.99a	0.89a
Celest®	46.00ab	18.75bc	7.675b	9.52a	1.19a

Table 4.3 Effects of *Bacillus cereus*1 and fungicides on infection, emergence and plant height of cowpea in *Pythium ultimum* inoculated soil

**B5B=*Bacillus cereus*1. *Value is a mean of four replications of 56 seedlings. Values in a column per trial followed by the same letter are not significantly different (*P = 0.05*).

4.2.3 *Fusarium solani*

With the exception of Apron® in the first and Celest® in the third trial, all treatments significantly increased emergence when compared to the inoculated control (Table 4.4). All treatments were able to increase emergence to the level of the uninoculated control in trial one and two, although only the biological control treatment (*B. cereus*1) achieved the same results in the third trial.

All treatments were able to reduce disease significantly in all trials when compared to the inoculated control. However, no treatment rendered complete control of *F. solani* (Table 4.4).

All treatments in all trials increased plant height and dry shoot and root mass when compared to the inoculated control except, for dry shoot mass in the Apron treatment in trial one and dry root mass of the *B. cereus*1, Subdue® and Celest® in treatment three. The plant height of, Apron® treated plants did not differ significantly from the uninoculated control, in trials one and two. Subdue® and Celest® also did not differ significantly from the uninoculated control in trial two. With regard to dry mass of shoots, all treatments gave results that were comparable to the uninoculated control in trial one, except Apron® (Table 4.4). In trial two, the shoot dry mass of plants treated with Apron® did not differ significantly from the uninoculated control. Similar results were also obtained with the *B. cereus*1 treatment in trial three. Plants treated with Apron® did not differ significantly from the uninoculated controls in terms of dry root mass in trial two and three. Likewise in trial three, plants treated with *B. cereus*1 and Celest® had dry root masses that were comparable to the uninoculated control.

4.2.3.1 Symptoms

Small brown lesions were observed at harvesting on the roots of plants grown in *F. solani* inoculated growth media and infected seedlings also showed root rot. There

was a reddish discolouration over the entire belowground stem and root system. Soft, dark brown or black cankers developed on the stem nodes and these often girdled the stem during disease development (Figure 4.3).

Figure 4.3. Disease symptoms caused by *Fusarium solani* on cowpea seedlings (a); and non-infected seedlings (b).

Treatments	Emergence (%)	Diseased seedlings (%)	Plant Height (cm)	Dry Shoot Mass (g)	Dry Root Mass (g)
Trial 1					
Inoculated control	44.25a*	47.25d	6.38a	8.90a	2.03a
Uninoculated control	64.75b	0.00a	9.48c	15.70bc	6.58c
B5B**	71.00b	17.2bc	7.73b	17.20bc	3.65b
Apron®	59.25ab	12.55b	8.48bc	12.55ab	3.60b
Subdue®	70.00b	17.70bc	7.98b	17.70c	3.35b
Celest®	68.75b	17.35bc	7.93b	17.35c	3.90b
Trial 2					
Inoculated control	45.75a	58.50d	8.750a	8.50a	1.93a
Uninoculated control	82.25b	0.00a	17.25c	23.50c	8.73c
B5B	74.50b	27.50c	14.00b	17.25b	4.93b
Apron®	71.0b	18.25cb	15.25cb	9.00cb	8.28c
Subdue®	78.75b	12.00b	14.25cb	15.75b	5.43b
Celest®	76.25b	13.50b	15.75cb	16.00b	4.75b
Trial 3					
Inoculated control	42.75a	64.78c	5.50a	5.86ab	0.55ab
Uninoculated control	77.50c	0.00a	11.10d	15.01e	2.45c
B5B	71.25c	34.38b	8.325cb	13.12de	2.38bc
Apron®	52.25b	31.25b	8.95c	10.73dc	2.63c
Subdue®	53.00b	39.58b	8.60cb	4.07a	0.34a
Celst®	49.00ab	33.30b	7.625b	8.21bc	1.47abc

Table 4.4 Effects of *Bacillus cereus*1 and fungicides on infection of cowpea in *Fusarium solani* inoculated soil

**B5B=*Bacillus cereus*1. *Value is a mean of four replications of 56 seedlings. Values in a column per trial followed by the same letter are not significantly different (*P = 0.05*).

CHAPTER 5

DISCUSSION

In the present study it was found that *B. cereus*1 was capable of reducing the mycelial growth of *R. solani* and *F. solani* but not *P. ultimum in vitro*. Similar results were reported by Wang *et al*. (2003), where some *Bacillus* spp. were found to be antagonistic against *R. solani*, *Fusarium* spp. and *P. ultimum* on pea. Sounto *et al*. (2004) reported that some *Bacillus* spp. showed strong antagonistic activity by reducing the mycelial growth of pathogenic fungi. In the current study *P. ultimum* grew at the same rate as the control. These results conflict with the findings of Wang *et al*. (2003), who reported that most species of *Bacillus* are antagonistic against *P. ultimum*.

Korsten and De Jager (1995) reported that *Bacillus* spp. was able to reduce mycelial growth of various pathogens of avocado. The results found in the present study also concur with the observations made by Ryder *et al*. (1998), who found that *B. cereus* inhibited mycelial growth of *R. solani,* a pathogen of wheat. Zaccardelli *et al*. (2003) and Martinez-Espinoza *et al*. (2004) reported that some *Bacillus* spp. are capable of reducing the mycelial growth of *R. solani* and *F. solani* because of their highly antagonistic activity. Harris *et al*. (1994) also reported that *Bacillus* spp. are capable of reducing the mycelial growth of *R. solani* supporting the findings of the current study where the pathogen was significantly reduced by *B. cereus*1.

Recent research has shown similar results on the capability of the bacteria to reduce mycelial growth of *F. solani* (Sounto *et al*., 2004). Various mechanisms are involved in antagonism, which include the production of antifungal substances, both water soluble and volatile (Ryder, 1998). In this study it appears that the *B. cereus*1 may have produced highly inhibitory substances that reduced the mycelial growth of *R. solani* and *F. solani*.

Apron® (metalaxyl) is a low-rate phenylamide systemic fungicide registered for the control of damping-off and seed rot diseases and is considered to be highly effective when used as a seed treatment against fungi belonging to the class Oomycetes (http://www.syngenta.co.za). Fisher & Hayes (1982), Ioannou & Grogan (1984), Whang & Kim (1995) and Babadoost & Islam (2003) reported *in vitro* reduction of mycelial growth of *Phytophthora* by Apron®. Harris & Nelson (1999) also conducted an *in vitro* experiment and found that Apron® is capable of inhibiting the mycelial growth of *R. solani*. The current study indicated that Apron® significantly reduced the mycelial growth of *R. solani*, *P. ultimum* and *F. solani* from the third to the ninth day. The fact that Apron® is highly effective against Oomycetes is supported by the findings of the current study, which demonstrated that the fungicide was capable of reducing the mycelial growth of *P. ultimum*.

Subdue® (mefenoxam) is registered for the control of *Pythium* and *Phytophthora* spp. (http://www.syngenta.com). Malvick & Gruden (2004) previously reported that Subdue® could reduce mycelial growth of *Phytophthora* spp. and Fravel *et al*. (2005) found Subdue® to be effective in reducing the mycelial growth of *Fusarium oxysporum*, the causal pathogen of wilt of tomatoes. Bucher & Pedersen (2004) also found that Subdue® could reduce the mycelial growth of *R. solani* of soybean. Subdue® (mefenoxam) significantly reduced mycelial growth of the pathogens *R. solani*, *F. solani* and *P. ultimum*, but was most effective against *P. ultimum*. The results found in the current study showed that the fungicide could be used for control of seedling diseases caused by all three pathogens. Thus, it was also confirmed that this fungicide could reduce the mycelial growth of pathogens belonging to classes other than Oomycetes.

Celest® (fludioxonil) is registered for the control of *Pythium* spp., *Fusarium* spp. and other seed rot fungi (http://www.Syngenta.co.za). Errampalli (2004) tested Celest® against blue mould of apples and reported that the fungicide was capable of reducing mycelial growth of *P. ultimum*. Celest® was reported to be effective in reducing mycelial growth of *Sclerotinia* causing drop in lettuce (Matheron & Porchas, 2004) and *Fusarium* spp., and controlling soybean diseases (Mueller *et al*., 2002),.

(Munkvold & O'Mara, 2002; Wang *et al*., 2005) and *R. solani*, a pathogen of soybean (Bucher & Pederson, 2004). Similarly the *in vitro* test in this study showed that Celest® could significantly reduce the mycelial growth of the pathogens *R. solani*, *P. ultimum* and *F. solani*.

The application of a biological control agent with the purpose of reducing disease incidence and providing optimal growth of seedlings was confirmed during this study. In the greenhouse trials the biological control agent (*B. cereus*1) reduced diseases caused by *R. solani*, *F. solani* and *P. ultimum* on cowpea. Complete control was obtained only for *P. ultimum* during the third trial. Application of *B. cereus*1 also contributed to the increase in seedling emergence and shoot length, resulting in high dry root and shoot masses which indicates that the bacteria could be used to stimulate growth of cowpea seedlings.

Bacillus spp. are known to be highly antagonistic against soilborne pathogens (Stevens *et al*., 2003) and several other pathogenic fungi (Batista *et al*., 2002; Wang *et al*., 2003). *Bacillus cereus* was capable of reducing diseases caused by the pathogen *Helminthosporium solani* (Martinez-Espinoza *et al*., 2004), and other root rot pathogens (Osburn *et al*., 1995), resulting in increased yield.

In this present study it was found that *B. cereus*1 when applied as a drench during planting, could reduce the disease incidence caused by all three pathogens. These results concur with the findings of Pleban & Ingel (1995), Ryder (1998) and Zheng & Sinclair (2000), who found that some *Bacillus* strains reduced diseases caused by *R. solani*. A similar observation was made by Zaccardelli *et al*. (2003), who reported that *Bacillus cereus* was capable of reducing diseases caused by *R. solani* and *F. solani* on potato. Sounto *et al*. (2004) found that some of the *Bacillus* spp. are capable of reducing diseases caused by *F. solani* and other damping-off pathogens. *Bacillus cereus* was also reported to be effective in reducing the diseases caused by *Phytophthora* spp. (Handelsman *et al*., 1990; Li *et al*., 1997; Jacobsen *et al*., 2004), the take-all pathogen of wheat (Ryder, 1998), and damping-off of alfalfa (Silo-Suh *et al*., 1994).

Apron® (metalaxyl) fungicide is used to provide full protection to the seed and seedlings against seedling diseases during the growing period (http://www.syngenta.co.za). The fungicide has previously been found to be effective against most of the diseases caused by *Phytophthora* spp. as reported by Farih *et al*. (1981), Whang & Kim (1995), Peters *et al*. (2001) and Malvick & Grunden (2004). Bhaskar *et al*. (2005a) reported similar results for the ability of Apron® to reduce the diseases caused by *F. solani*. Mixtures of Apron®, Celest® and difenoconazole are effective in reducing diseases caused by *Fusarium* root rot (Wang *et al*., 2005). Fisher & Hayes (1982) and Brantner & Windels (1998) also reported Apron® to be effective in reducing diseases caused by *P. ultimum*. Keinath *et al*. (2000) reported that Apron® alone or in combination with carboxin can effectively control the disease caused by *R. solani* on snap bean. Apron® plus Celest® were also found to be effective in reducing *R. solani* on cucumber (Ghate, 1991). In the present study it was found that Apron® (metalaxyl) could be used to reduce diseases caused by *R. solani*, *F. solani* and *P. ultimum*. However, complete control of all the pathogens in all trials was not obtained. During harvesting, some of the seedlings showed minor symptoms, which indicates that they were protected to some extent from the damage caused by the pathogens. Increased emergence and shoot length resulting from the application of the fungicide indicated that the fungicides do not only protect the seed and seedlings against pathogens, but also play an important role in promoting growth of the plant.

Subdue® (mefenoxam) can be used for the control of seedling diseases as reported by several authors. However, the fungicide is known to be highly effective when used as a seed treatment (http://www.syngenta.co.za). Martinez-Espinoza *et al*. (2004) found that the Subdue® was effective in reducing *R. solani* infection on ornamental plants. Subdue® has been found to be highly effective against *Pythium* spp. causing seedling damping-off of pumpkin (Babadoost & Islam, 2003) and downy mildew (Kirk, 2003). Chase (1999) and McGovern *et al*. (2001) also found Subdue® to be capable of reducing the damage caused *Fusarium* species. In the present study it was found that the Subdue® could reduce the diseases caused by *R. solani*, *P. ultimum* and *F. solani* but did not give complete control of these pathogens and this might be because it is mostly active against the Oomycetes viz. *Pythium* and *Phytophthora* (Cohen, 1986).

Celest® (fludioxonil) is used as a seed treatment for the control of seed and soilborne diseases which are considered to cause serious damage to many crops as they attack the crop in early stages (http://www.syngenta.co.za). Celest® in combination with Subdue® has been found to be effective against *R. solani* on soybean (Bucher & Pedersen, 2004) and in reducing diseases caused by *Rhizoctonia* root rot of ornamental plants. Celest® is also effective in controlling *Fusarium* spp. on maize (Munkvold & O'Mara, 2002) and other *Fusarium* diseases when combined with biological control agents (Wang *et al*., 2005). The fungicide also reduces diseases caused by *Phytophthora infestans* on potatoes (Inglis *et al*., 1999), *Pythium* spp. and *R. solani* (Mazzola, 1998). In the current study the fungicide Celest® (fludioxonil) was able to reduce diseases caused by all three pathogens (*P. ultimum*, *F. solani* and *R. solani*) in all trials. Although the fungicide is registered for the control of all three pathogens, complete control of all three was not obtained but increase in emergence and shoot length of the seedlings indicates it can be used for growth promotion and seed protection.

Having the first application of the fungicides on the 14th day after planting is not recommended, especially for the control of diseases that could damage the seeds before germination. It is suggested that the fungicides be applied immediately after planting or as a seed treatment. The current study revealed that Subdue® and Apron® could be used to reduce diseases caused by *F. solani* and *P. ultimum*. Celest® was found to be effective in reducing the diseases caused by all three pathogens. However, none of the three fungicides gave complete control of any of the pathogens. In the present study it was found that *B. cereus*1 could be used for the control of diseases caused by all three pathogens, especially when applied a day after planting.

CHAPTER 6

GENERAL CONCLUSION

Summerfield & Roberts (1985) reported that cowpea is vulnerable to many diseases that cause severe damage, leading to low yields during harvest. Seedling diseases caused by pathogens such as *Fusarium solani*, *Rhizoctonia solani* and *Pythium ultimum* cause extensive losses as they attack cowpea at an early stage (Berland *et al*., 2003). Various chemicals and a biological control agent were screened to test their effectivity against these seedling diseases on cowpea *in vitro* and *in vivo* in the greenhouse.

The *in vitro* tests were conducted using the chemicals Apron® (metalaxyl), Celest® (fludioxonil) and Subdue® (mefenoxam) and a biological control agent (*B. cereus*1). From this experiment it was found that all fungicides were able to reduce mycelial growth of *P. ultimum*, *R. solani* and *F. solani* (Chapter 4). *Bacillus cereus*1 significantly reduced the mycelial growth of *R. solani* and *F. solani* but not *P. ultimum*. The reason for the bacteria failing to reduce mycelial growth of *P. ultimum* is not fully understood, however, it might be because of this *Pythium* species' ability to grow fast, making it difficult for the bacteria to colonise it.

The *in vivo* greenhouse study revealed that application of the fungicides and the biological control agent could reduce the disease incidence caused by *P. ultimum*, *F. solani* and *R. solani* (Chapter 4). However, all fungicides failed to give complete control of the pathogen. This may be due to the fact that they were only applied on the 14th and 28th days after planting. Therefore it should be recommended that the fungicides be applied sooner after planting, so that they can control the diseases during seedling emergence as well as seed germination. During harvesting some of the seedlings showed minor lesions on the stems and roots, which indicates that they

were protected to some extent from the damage caused by the pathogens. It is therefore recommended that all three fungicides be applied during sowing, or seed be treated with fungicides such as thiram so that they can protect the seeds during germination. It was also found that the bacterium *B. cereus1* is capable of reducing the diseases caused by all three pathogens tested. Although the biological control agent did not reduce the mycelial growth of *P. ultimum* during the *in vitro* assay, the greenhouse trials showed promising results. Further studies are required on the effectivity of *B. cereus*1 against seedling diseases. All fungicides reduced mycelial growth and diseases of all the pathogens during both *in vitro* and *in vivo* experiment respectively. During the greenhouse trials the biological control agent controlled all pathogens better than the fungicides.

In conclusion, there is a need for future research on the application of these three fungicides and their effectivity against seedling diseases. Based on the findings during this study it can therefore be recommended that the fungicides be applied immediately after planting or seed treatment such as thiram be used before applying the fungicides. Celest® also significantly reduced diseases caused by *R. solani*, *F. solani* and *P. ultimum*, when compared to the inoculated control. However, during the *in vitro* trial all fungicides reduced the mycelial growth of *F. solani* to the level of the uninoculated control. Much attention should also be given to the use of biological control measures, as few of them have been registered for the control of seedling diseases. The bacteria showed antagonistic behaviour against all three pathogens, however, it only gave a high level of control of *P. ultimum*, only in the third trial. From these results it can also be recommended that *B. cereus*1 be applied twice during the seedling emergence period, firstly during germination and the second application could be made few days after emergence.

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