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**Evaluation of three fungicides for control
of soilborne diseases of lettuce seedlings**

By

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**Submitted in partial fulfillment of the requirements for the
Degree of**

M Inst Agrar (Plant Protection)

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Natural and Agricultural Sciences**

University of Pretoria

Pretoria

March 2008

DECLARATION

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

J.B. Kalonji Kabengele

ACKNOWLEDGEMENTS

Professor Theresa Ann Sheila Aveling (Terry) my supervisor let me thank you for your acceptance to lead me in a difficult road of research.

Professor N. Labuschagne and Dr. J. van der Waals my co-supervisors, I will never stop thinking about your support, scientific advice and encouragement.

Terry; Jays my sons and baby Jayde this thesis must be for you a shining model to follow in this world.

Ambroise Kalonji and Aimerance Mukundi I am proud to have you as my parents, this study is a proof and a symbol of my fidelity to you.

V. Govender Z. Pieterse , M. Ramusi. D. Kandolo, Lorna Kheumbo, Besrat and Muya Claude my colleagues you never abandon me. Thanks for your valuable support for the completion of this thesis.

Fi-Amore-FiFa, Thanks for being with me all the time,

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SUMMARY

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Co-Supervisors: Professor N Labuschagne

Doctor J. E. Van der Waals

Lettuce (*Lactuca sativa* L.) seedlings diseases caused by soilborne pathogens are characterised by root rot, stem rot and damping-off of the seedlings that can occur at any time during growth. *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* are known to be the important destructive pathogens of lettuce, causing severe yield losses in South Africa. The aim of this research was to evaluate the effects of three selected fungicides to control these pathogens on lettuce seedlings.

In this study the fungicides metalaxyl (Apron[®]), fludioxonil (Celest[®]) and mefenoxam (Subdue[®]) were applied at two concentrations as single and double doses on lettuce seedlings to determine their efficacy to control the pathogens *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* after significant reduction of mycelia growth was observed *in vitro*.

Cultures of *P. ultimum* (UPGH024), *R. solani* (UPGH122) and *F. solani* (UPGH122) were obtained from the culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria and cultivated on PDA for 2 days at 25°C. Pasteurised soil was artificially inoculated with these pathogens. For the first experiment lettuce seeds were planted in polystyrene seedling trays at a depth of 1.0 cm. There were four replications of 50 seeds per treatment. In Experiment 2 pots (12 cm x 7 cm) were filled with pasteurised growing medium and 3-week old seedlings were transplanted. There were three replications of six pots containing three plants each. Seedling trays and pots were drenched with fungicides and placed in a randomised block design in a controlled environment room at 20-26°C with a 12h-light/dark regime. The seedling trays and pots were rotated daily in the room. Seedling trays and pots were watered daily to maintain field capacity.

The seedlings were able to grow larger in the pots than in seedling trays. It was confirmed that the treatment with fludioxonil (Celest[®]) at double and single dose inhibited the growth of the three fungi *F. solani*, *P. ultimum* and *R. solani* on lettuce seedlings without causing phytotoxicity. All three fungicides significantly reduced the diseases caused by the three

pathogens. These findings are consistent with previous reports that fludioxonil, metalaxyl and mefenoxam can control oomycete fungi. There are few registered fungicides for the control of *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* on lettuce, therefore further work will aim to confirm these results in the field.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Background Introduction

Lettuce (*Lactuca sativa* L.) is an annual herbaceous plant belonging to the Compositae (Asteraceae) family (Romani *et al.*, 2002). Lettuce is an important crop and is the most consumed salad vegetable in many countries (Nicolle *et al.*, 2003). Its use has continued to increase in salad bars and fast foods, because of its long storage life, good quality and its perception as being a healthy food (Ferrerres *et al.*, 1997). Lettuce differs from other vegetables in the content of several types of micronutrients and fibres. In addition, lettuce contains appreciable amounts of water-soluble antioxidants such as vitamin C and lutein (Nicolle *et al.*, 2003). Recently in medicine, *Lactuca* has been reported to prevent chronic diseases such as cancer and its seeds are used for the treatment of inflammation and asthma (Pavlou and Vakalounakis, 2005). In addition, lettuce residues in the soil can control root and stem rot diseases of cucumber caused by *Fusarium oxysporum* Schlechtend.: Fr f.sp. *radicis-cucumerinum* (Pavlou and Vakalounakis, 2005).

Economically significant root pathogens that cause damage to lettuce include *Fusarium solani* f.sp. *phaseoli* Mart, *Rhizoctonia solani* Kühn and *Pythium ultimum* var *ultimum* Trow. These soil-borne pathogens are major problems for greenhouse production; causing poor plant stands and stunted growth (Mercier and Manker, 2005). Vegetables and other nursery plants produced from seed can be affected by such pathogens resulting in damping-off, root rot, stem rot and mortality or lower quality of propagating plant material (Mercier and Manker, 2005).

1.2 Motivation for the study

Strategies to control soilborne diseases such as *F. solani*, *R. solani* and *P. ultimum* are limited because of their ecological behaviour and the high rate of resistance to the control measures under different environmental conditions (Agrios, 2005). New fungicides to protect plants from soilborne pathogens are constantly in demand.

Chemical seed protectants and soil-applied fungicides have been recommended for the control of soilborne diseases, but have not been completely effective and can also cause phytotoxicity

and negatively affect growth (Estevez de Jensen *et al.*, 2002; Spadaro and Gullino, 2005). Metalaxyl (17% active ingredient) has also been used as a seed treatment for root rot, but has limited activity and does not provide effective protection for the growing root (Estevez de Jensen *et al.*, 2002). Biological control agents rarely provide the level of protection that is equal to that obtained with chemical fungicides (Elmer and McGovern, 2004). Steam sterilisation in some cases is not economically viable and the use of methyl bromide is banned in many countries so there is a need to find a new alternative means of control (Gül *et al.*, 2005).

1.3 The aim of the study

The aim of this study was to evaluate the fungicides: metalaxyl (Apron[®] XL); fludioxonil (Celest[®] XL) and mefenoxam (Subdue[®] MAXX) at two concentrations (single and double dose) for the control of the pathogens *Fusarium solani*, f.sp. *phaseoli*, *Rhizoctonia solani* and *Pythium ultimum* var *ultimum* on lettuce seedlings.

1.4 Objectives

The objectives of the study were:

- a) To optimise fungicide requirements for effective control of seedling diseases caused by *F. solani*, *R. solani* and *P. ultimum* on lettuce in the greenhouse,
- b) To evaluate the efficacy of the above mentioned fungicides against fungal seedling diseases of lettuce, and
- c) To assess phytotoxicity, stunting and growth effects due to application of fungicides tested.

CHAPTER 2: LITERATURE REVIEW

2.1 The host: lettuce

2.1.1 Introduction

Lettuce (*Lactuca sativa* L.) is the most widely used leaf vegetable in salads and as garnishes (Gender, 1976; Dekker, 1999). It is among the most important salad crops, whether grown for home use or for the market (Gender, 1976).

Due to its considerable diversity, genetically and in terms of scale and means of production (Caten, 1987) this crop has been developed in many varying forms. These include cold-tolerant varieties for mid-winter production and snow bolting, and heat-tolerant varieties for summer and autumn growing (Janick, 1978). In addition lettuce is cultivated all year round because it grows both outside and in the glasshouse (de Vries, 1997). Lettuce has been the subject of many research projects since 1917 and its consumption has increased enormously due to its health-giving properties (Edmon, 1959).

2.1.2 Origin

There are several different opinions about the centre of origin of cultivated lettuce (de Vries, 1997). According to George (1989), salad lettuce is generally thought to be derived from the wild species of *Lactuca serriola* L., native to Asia Minor. Jules (1974) reported that the wild species *Lactuca serriola* was grown by Persia's king in 500 B.C. and originated in the Middle East Asia. According to de Vries (1997), the domestication of lettuce took place in South West Asia. According to Lindqvist (1960), lettuce has its origin in the Kurdistan-Mesopotamia area and not in Egypt. This opinion seems acceptable because the highest number of wild lettuce species is found between the Euphrates and Tigris rivers (de Vries, 1997).

2.1.3 Nomenclature

Lettuce is an annual crop, belonging to the Asteraceae family, some common names are: lettuce (English); laittu (French); blaarslaai (Afrikaans).

2.1.4 Botanical description

Lettuce consists of two main groups:

- a) Cabbage type with broad, wrinkled leaves, densely over-lapping and usually forming a dense “heart” and
- b) Cos type with light green leaves, elongated and not wrinkled; usually form a heart except under high temperatures (Tindall, 1979).

Tindall (1983), Kalloo (1988) and Dekker (1999) described lettuce as a glabrous herb and an annual plant. Depending on varieties the colours of lettuce seeds are black, yellow and grey, the leaves are almost sessile, arranged spirally in a rosette and varying in size (10-25 cm in length). Colours range from yellow, green or blue and the degree of colouring ranges from light or medium to dark. In maturity leaves are lustre.

According to de Vries (1997), the edible genotypes have been arranged in six lettuce cultivar groups namely Butter head, Cos, Latin, Crisp head, Cutting and Stalk lettuce.

2.1.5 Cultivation conditions

Lettuce is easy to grow and different varieties are suited to be grown during different seasons of the year (Healy, 1995). When lettuce is grown in high temperatures, the crop usually bolts (Janick, 1978). Tindall (1983) and Lee (2000) found that lettuce is a temperate crop that normally grows under relatively cool climatic conditions. It can be grown on sandy loam soil (Schery, 1954). The cultivation of lettuce is adapted to specific environments; there are wide variations in soil types, temperature, day length, water quality and relative humidity, particularly among major areas (Bassett, 1986).

Lettuce is relatively susceptible to drought damage, but can be grown successfully in humid regions where precipitation is variable (Wilks and Wolfe, 1998). In addition, the best head development takes place at lower soil temperatures and the general development at higher temperatures (Nothmann, 1977). For a high yield, it is advisable to choose specific varieties for each season (Mbey, 1978). In the southern and southwestern Cape regions of South

Africa, lettuce can be planted throughout most of the year (Southwood, 2004) and can be grown in most soil types. However for optimum growth, heavier soils with the soil pH 5,8 - 6,5 are preferable. Nothmann (1977) and Southwood (2004) found that warming the soil to 13 or 18°C accelerated the maturation of the lettuce head.

Lettuce is tolerant to a wide range of soil conditions and can be grown in most tropical countries (Tindall, 1983). It is widely grown in Malaysia, Africa, America, Philippines, and most tropical countries (Tindall, 1979).

The essential pre-condition for successful growth is a well-mulched, humus-rich bed, with well-drained soil and old manure (Healy, 1995). Dry soils normally cause bolting, but lettuce can be grown for many years without irrigation (Fogg, 1976). Excessive moisture followed by rainfall may cause crop damage (Wilks and Wolfe, 1998). Lettuce also gives good yields in gravel, hydroponics and/or aeroponic systems (Resh, 2001). However, the hydroponics system has two principal disadvantages: the high costs of capital and energy inputs, as compared to open-field agriculture. Pricing studies have revealed that only high-quality, garden-type vegetables such as lettuce, can provide suitable returns on investment (Healy, 1995).

2.1.6 Planting and harvesting procedures

Lettuce is eaten all year round because it is cultivated both outside and in the glasshouse (de Vries, 1997). The optimal planting date varies according to cultivars, climatic conditions and soils. The best planting times in South Africa are from January to April and from March to July (Kirsten, 1982). The seeds can be sown every fourteenth day to give a succession of crops through to November (Dick, 1982), however the seeds of hard varieties can be sown in September to early October (Kirsten, 1982). Lettuce seeds are light and fine and considerable care should be taken during sowing in seedbeds. The seeds can be scattered at a rate of 1 g seed per m² (Dekker, 1999).

The cultivation of lettuce in hydroponic systems can supply the market all year round (Resh, 2001). According to Reichardt (1993) and Resh (2001), lettuce responds well in intercropping culture and the yield is high when cultivated with crops such as bush beans, dill, peas, cabbage, leek, tomato, onion and beetroot.

2.1.7 Uses of lettuce

There are some less common uses for lettuce (Bassett, 1986), but it is mostly widely used as a leaf vegetable in salads whilst seeds can be used for production of oil (de Vries, 1997; Dekker, 1999). A cigarette containing no nicotine is made from lettuce leaves (Bassett, 1986). Lettuce debris incorporated into soil offers new options for controlling root and stem rot diseases of cucumber especially if combined with other methods (Pavlou and Vakalounakis, 2005).

The chemical composition of lettuce corresponds with that of most edible vegetables (Table 2.1) and the use of lettuce leaves provides a source of proteins, vitamin A, water, calcium and potassium (Tindall, 1983; Dekker, 1999). In addition, lettuce contains other nutrients such as Vitamin B and C and carotenoids that contribute to improved protection against cardiovascular diseases (Nicolle *et al.*, 2003). Lettuce seeds also contain vitamin E (de Vries, 1997).

Table 2.1: Nutritional composition of lettuce in 100 g foodstuffs

Compound	Lettuce leaves (100 g)
Protein	0.8-1.6 %
Lipid	0.1-0.2 %
Sugars	1,2-2.1 %
Water	98 %
Calcium	13-36 %

Source: (Tindall, 1983; Dekker, 1999).

2.2. Major lettuce diseases

Lettuce can be contaminated during growth from many sources such as soil, water, wild animals, birds and insects in the field. In addition, after production, processes involving harvesting, washing, cutting, packing and shipping can also cause contamination (Koseki and Isobe, 2005).

2.2.1 *Fusarium solani*

2.2.1.1 Introduction

Fusarium species play an important role as plant pathogens, causing a wide range of diseases such as vascular wilt and stem rot in a diversity of hosts (Schollenberger *et al.*, 2005). The group of *Fusarium* fungi is important as plant pathogens in fields and as mycotoxin producers during processing and storage (Falasconi *et al.*, 2005). *F. solani* is a soil saprophyte and soilborne plant pathogen, which is responsible for a variety of seedling diseases including seed rots, pre-and post-emergence damping-off, root rot and late season damping-off (Duarte *et al.*, 1998; Allen *et al.*, 2004).

2.2.1.2 Origin, taxonomy and distribution

Fusarium spp. may have been one of the first fungi to become established on earth (Nelson *et al.*, 1981). The genus *Fusarium* is among the most heterogeneous fungal genera and classification of species within this genus is very difficult (Llorens *et al.*, 2006).

However, *Fusarium solani* is one of the few *Fusarium* species that is easily identifiable. The genus is subdivided into ten *formae specialis* (f.sp.) based on host range tests (Nelson *et al.*, 1981; Vitale *et al.*, 2004).

The fungus was described and clearly illustrated by Martius in 1842, as *Fusisporium solani*. Forty years later, in 1881, Saccardo renamed it *Fusarium solani* (Booth, 1971). The genus *fusarium* is imperfecti fungi (Deuteromycotina), and belongs to the Kingdom fungi, order Hypocreales, Family Hypocreaceae (Fry, 2004). The survival of *F. solani* in the soil depends on the production of chlamydospores, which are resistant structures capable of survival in the absence of the host plant (Schipper and Old, 1973).

Most *Fusarium* strains occur worldwide (Schollenberger *et al.*, 2005). The distribution of *Fusarium* species is influenced by weather conditions such as temperature and humidity (Kosiak *et al.*, 2004).

2.2.1.3 Morphology and cultural characteristics

The genus *Fusarium* is characterised by the production of septate, hyaline, delicately curved elongate macroconidia (Moss and Thrane, 2004). *F. solani* germinates in cultivated soils in the rhizospheres of host or non-host plants (Mondal *et al.*, 1996). According to Song *et al.* (2004), the use of hydroponics cultivation systems in greenhouses also offers favourable conditions for *F. solani*. *F. solani* f. sp. *phaseoli* has no specificity for nutrients of crop plants for germination (Mondal *et al.*, 1996).

2.2.1.4 Epidemiology, ecology and environmental conditions

According to Mondal *et al.* (1996), *Fusarium solani* germinates in cultivated soils in the rhizospheres of host or non-host plants. *F. solani* develops most rapidly at temperatures ranging from 24-29°C. The effects of temperature on propagule density may influence the survival and the life cycle of *F. solani* (Seremi *et al.*, 1999). The fungus is spreaded by infested plants and soil on farm machinery, drainage water and boots (Jones, 1997). Koseki and Isobe (2005) also reported that vegetables could be contaminated during growth from many sources such as soil, water, wild animals, birds and insects.

When a susceptible crop is present, chlamydospores germinate and the fungus penetrates the plant through young roots or stems. The mycelium enters the xylem and progresses up the root and stem into the leaves (Jones, 1997).

The majority of *Fusarium* species are normally found in or on soil, where they exist as colonizers of living plants or plant residues within the soil (Nelson *et al.*, 1981). This pathogen can be found notably in the prairie soils, but is missing or rare in forest soils (Griffin, 1972). Furthermore, *Fusarium* can remain viable for up to 30 years (Thangavelu *et al.*, 2003). *Fusarium solani* levels in the field depend on the temperature and other factors such as rainfall. In South Africa *F. solani* is more abundant in warmer regions (Seremi *et al.*, 1999).

2.2.1.5 Symptoms

Fusarium solani diseases are characterised by distinct foliar symptoms that develop after infection at any growth stage. *Fusarium oxysporum* Schl.f.sp. *lactucae* Matuo and Motohashi can affect the lettuce plant at all ages, from seedlings to mature plant (Hubbard and Gerik, 1993). The most conspicuous symptoms begin with chlorotic mottling followed by interveinal chlorosis and necrosis (Shuxian *et al.*, 2000). Besides these foliar symptoms, roots are rotten and the vascular system of lower stems is discoloured and reduced (Rupe *et al.*, 1997).

The first symptom in the field is wilting of the leaves, within several days after infection, followed by plant death. Generally *Fusarium* is limited to the crown area of the plant; the taproots are not affected, except under extremely wet conditions (Martyn, 2000). On tomato, yellowing occurs along the margins of the oldest leaves, followed by necrosis and collapse of the leaf petiole (Jones, 1997). Soon after yellowing appears on the lettuce seedling, the water conducting tissue (xylem) becomes reddish brown, usually first on one side of the stem. Other plants such as tomato may wilt slowly and still be alive at the end of the harvest (Jones, 1997).

2.2.1.6 Control of *Fusarium* spp.

Crop rotation can be effective for controlling a number of soilborne diseases including *Fusarium solani* f.sp. *phaseoli*. These pathogens are observed in greenhouses with soil that has been disinfested with broad-spectrum biocides. In a greenhouse which had intense damage to cucumber in the past, control of this pathogen was attempted using calcium cyanamide (100 g/m superior 2), organic matter in the form of dried shredded wheat straw, and soil solarization for three or six weeks under transparent polyethylene sheets (0.05 mm thick), in all possible combinations. Combined treatments of calcium cyanamide, solarization and shredded wheat straw showed the greatest reduction of soil population of the pathogen (over 99% reduction) (Bourbos *et al.*, 1997).

Chemical control remains the main measure to reduce disease incidence in various vegetable crops. The application of antimicrobial chemicals belonging to the benzimidazoles, aromatic

and hydrocarbons, and sterol biosynthesis inhibitors is used for treatment of disease caused by *Fusarium* spp. (Dimitra *et al.*, 2002).

Biological control of soil-borne pathogens may resolve problems posed by fungicides. Antagonistic fungi are known to compete with disease-producing fungi and affect their spread (Abada, 1994). Biological control of *Fusarium* root and stem rots has been attempted with some success by incorporating organic materials such as barley, straw, lettuce and chitin in the soil, thus favouring the increase of several fungal and bacterial antagonists, mycorrhizal fungi, or antagonistic *Pseudomonas* bacteria (Agrios, 2005). Treatment of propagative stock with benomyl or application of benomyl sprays on the plant in the field or greenhouse has helped reduce the incidence of *Fusarium* root rot on some types of plants (Nelson *et al.*, 1981; Agrios, 2005).

2.2.2 *Pythium ultimum* var *ultimum*

2.2.2.1 Introduction

Seed rot and damping-off due to *Pythium ultimum* var *ultimum* Trow is a serious disease of a wide range of seedlings in nurseries, glasshouses and gardens (Harris and Adkins, 1999). Worldwide in occurrence, it is the cause of considerable economic loss, particularly in high-value horticultural and arboricultural industries (Thomson and Burns, 1989; Appiah *et al.*, 2005).

Pythium ultimum is a devastating pathogen that can survive under humid and warm conditions and may induce damping-off, root rot, crown and lower stem rot, or storage rot on a wide variety of crop species including lettuce (Cherif *et al.*, 1991; Benhamou and Brodeur, 2001). The suppression of *P. ultimum* has been demonstrated to be effective, and in some cases ineffective, according to different management processes for reducing disease incidence including the use of fungicides, detergents and biological control agents (Hultberg *et al.*, 2000; van Os and van Ginkel, 2001).

2.2.2.2 Origin, taxonomy and distribution

According to Paul (2003), *Pythium* is a genus in the class oomycetes in the Kingdom Chromista. *Pythium* belongs to the family of Pythiaceae, order Peronosporales. *Pythium*

contains species that range from saprophytic to facultative parasites with limited host ranges (Tambong *et al.*, 1999).

It is often difficult to identify *Pythium* species and pathogenic strains on the basis of morphological traits (Tambong *et al.*, 1999). Van der Plaats-Niterink (1981), as quoted by Martin (1990), classified *Pythium* spp. according to the types of sporangia or sporangium swellings produced. Species were mainly identified by the study of their morphology such as the size and shape of oogonia, antheridia and sporangia (Paul *et al.*, 2005). *Pythium* spp. taxonomic references are based on comparison of morphological characteristics and temperature-growth relationships of different members of the genus; certain species are differentiated by quantitative differences, such as the size of oogonia, oospores and the number of antheridia per oogonium (Chen *et al.* 1991; Paul, 2001). Recently the morphological description of different species of *Pythium* has been complemented by molecular characteristics (Galland and Paul, 2001).

2.2.2.3 Morphology and cultural characteristics

Mihail *et al.* (2002) reported that the members of the genus *Pythium* are filamentous, heterotrophic microorganisms, which are ecologically and morphologically similar to the true fungi. These fungi are phenotypically true fungi having coenocytic branched mycelia (Galland and Paul, 2001). The majority of these organisms produce biflagellate zoospores (Paul *et al.*, 2005).

Pythium spp. can be classified according to morphological characteristics of the sexual and asexual reproduction structures or according to the types of sporangia and hyphal swellings produced (Martin, 1990). Suffert and Guibert (2007) reported that the identification of *Pythium* spp. using keys based on spore and sporangial morphology is difficult, because several species are asexual or heterothallic and do not readily produce diagnostic organs. However, in the last 10 years, molecular techniques have been developed for the identification of *Pythium* spp. (Paul *et al.*, 2000).

The typical morphology of the genus *Pythium* is a slow growing oomycete, having large spherical, globose to cylindrical sporangia, smooth-walled oogonia and mostly hypogynous antheridia (Paul *et al.*, 2005). Chen *et al.* (1991) found that the morphological features of

Pythium spp. are frequently variable and are influenced by environmental conditions. The oospores of *Pythium perplexum* H. Kouyeas are small (16 µm in diameter) compared to those of *Pythium ultimum*, which are 21 µm in diameter (Galland and Paul, 2001).

In general *Pythium* spp. are difficult to isolate from soil or plant material by the use of nutrient agar techniques (Cooper *et al.*, 2004). In contrast the fungus reproduces sexually by forming antheridia and oogonia on solid media as well as in water (Paul, 2001). Propagules of *Pythium* spp. depend on exogenous nutrients for growth (van Os and van Ginkel, 2001). The germination of sporangia of *Pythium ultimum* is rapid (1-3 h) (Paulitz and Baker, 1988).

2.2.2.4 Epidemiology, ecology and environmental conditions

Pythium spp. affect nearly every crop grown in every part of the world. The pathogen causes seed rot that kills seeds before their germination or may invade roots as well as stems, turning them brown leading to economical loss (Yephet ben-Yephet *et al.*, 1999; Roberts *et al.*, 2005). According to Sicard *et al.* (2003), *Pythium* spp. are responsible for losses of food crops and ornamental plants and the interaction between plants and oomycete pathogens remains largely unknown.

Oomycetes produce free-swimming zoospores, which are agents for dispersal and infection of new hosts (Appiah *et al.*, 2005). *Pythium* spp. are causal agents of several major plant diseases. They infect plants by means of zoospores that swim freely in water films in the soil or on plant surfaces (Morris and Ward, 1992). Since *Pythium* is not capable of penetrating undamaged periderm tissue, infection occurs almost exclusively through wounds (Taylor *et al.*, 2004). Growth or expansion of *Pythium* spp. in the soil is moderated by environmental factors such as moisture, temperature, soil pH, and the presence of specific soil minerals (Jones, 1997). The fungus infects the root tips of the host, and if favourable conditions continue, the fungus moves progressively up the root to the crown and stem (Cherif *et al.*, 1991)

Plant diseases caused by *Pythium* spp. are more prevalent in fields with wet soils than in fields with low soil moisture (Biesbrock and Hendrix, 1970). However, the severity of disease depends on the weather conditions, susceptibility of cultivars and the inoculum source. Some cultivars show a high level of *Pythium* root rot prevalent in cool, wet soil covered with crop

debris (Filonow and Dole, 1999; Agrios, 2005). *Pythium* spp. usually cause seedling diseases and pre-and post-emergence damping-off in a low temperature environment (Zhong *et al.*, 2000). Aveling *et al.* (2001) found that the incidence of damping-off and stem rot on cowpea was high in humid soil, moreover in their studies it was confirmed that the infection rate was highest at the seedling stage.

The members of the genus *Pythium* live as saprophytes occupying a wide range of terrestrial and aquatic habitats (Stredansky *et al.*, 2000) *Pythium* spp. can attack seeds and seedlings at all temperatures favourable for the germination of numerous crops including lettuce (Bardin *et al.*, 2003). Many oomycete plant pathogens especially several *Pythium* species, have optimal pathogenicity at 11°C (Pedersen *et al.*, 2003). A cool wet environment can slow seed germination and seed growth, and may be conducive for the development of damping-off.

Environmental conditions such as high soil moisture, and abundance of decomposing material are favourable for *Pythium* growth (Pankhurst *et al.*, 1995; Pedersen *et al.*, 2003). The sporangia of *Pythium* play an important role in the life cycle and ecology of this plant pathogenic fungus surviving in adverse conditions (Paulitz and Baker, 1988; Grosch *et al.*, 2005).

Pythium spp. are present in dust and soil mix particles on floors and walkways of greenhouses (Stephans *et al.*, 1983, as cited by Harris *et al.*, 1994). Lettuce seedling diseases occur most frequently under cool wet conditions immediately after planting (Filonow and Dole, 1999). In the temperature range of 20-30°C, rotting caused by *P. ultimum* can be serious in potatoes after harvest, but disease severity is low at <10°C (Lui and Kushalappa, 2003).

According to Sicard *et al.* (2003), *Pythium* may have distinct molecular mechanisms for interacting with plant hosts compared to fungi of other classes. However, oomycetes, including *Pythium*, and fungal pathogens, may exhibit similar modes of parasitism.

2.2.2.5 Symptoms

The symptoms or plant response may differ between plants since they depend on the metabolite produced by the pathogen, *Pythium* (Rey *et al.*, 2001). Adandonon (2000) reported that the symptoms of *P. ultimum* on cowpea are characterized by rot of the basal stem and

crown of plants and wilting. Similarly, Utkhede and Bogdanoff (2003) reported that the lower part of the plant becomes thinner than the upper part of young apple trees as the fungus grows. According to Chellemi *et al.* (2000), when pepper roots are affected they appear brown, and in later stages, the whole root mass becomes brown. The typical symptoms of *Pythium* damping-off of pepper are rotting stems and roots at or near the soil line (Chatterton *et al.*, 2004). When seeds of a susceptible plant are planted in infested soils the plants fail to germinate, become soft and mushy. The plants finally disintegrate; the most obvious symptoms above ground are gaps in the planting row caused by seed decay or seedling death (Agrios, 2005). Symptoms associated with *Pythium* root rot of pepper include wilt, lack of vigour, and nutrient deficiencies (Chellemi, 2006).

The genus *Pythium* may cause swelling behind the root tip and reduced root growth in cucumber. An intriguing phenomenon related to plants infected by *Pythium* is the considerable yield losses that can occur even in the absence of any obvious root necrosis or apparent symptoms (Moulin *et al.*, 1994; Schwarz and Grosch, 2003).

2.2.2.6 Control of *Pythium ultimum*

In nature the control of the pathogen is believed to be a complex phenomenon in which components of indigenous soil naturally regulate the growth of the pathogen (van Os and van Ginkel, 2001). However, the risk of damping-off can be reduced by good nursery hygiene (Harris *et al.*, 1994). *P. ultimum* in the greenhouse can be controlled through the use of soil sterilized by steam or dry heat, and through the use of chemically treated seed (Agrios, 2005). Fungicides such as captan and thiram that were registered for control of *Pythium* root rot in the greenhouse are not effective on crops such as cucumber (Utkhede and Bogdanoff, 2003). Metalaxyl and biological control agents have been successful in controlling *P. ultimum* in potatoes, but these are have not been commercialised (Lui and Kushalappa, 2003).

According to Sumner *et al.* (1995), organic amendments may reduce crop damage from soilborne pathogens in fields. Although fungicides have shown some promise in controlling *Pythium* in hydroponics, further manipulation of light, temperature and nutrient composition may also reduce the severity of disease (Paulitz *et al.*, 1992).

Sanitation, such as use of clean growing medium and plant material, is the primary way of preventing lettuce damping-off and other root diseases. Soil disinfestation, carried out through steaming or fumigation with methyl bromide, remains the most effective practice for the control of soilborne diseases in most vegetables (Nemec *et al.*, 1996; Mercier and Manker, 2005).

In view of the interactions between fungicides and different *Pythium* spp., Utkhede and Smith (1991) reported that fosetyl-Al and metalaxyl can suppress the pathogen *P. ultimum* without eradicating it, and that these two chemicals can be used as preventative rather than curative treatments in nurseries. On the other hand metalaxyl is fungistatic to *Pythium cactorum* (Leb and Cohn Schroet) *Pythium cambivora*, (Petri) Buism., but fungicidal to *P. ultimum*. In contrast fosetyl-Al at high concentrations was fungicidal to *Pythium cactorum*, *P. cambivora* and *P. ultimum* (Utkhede and Smith, 1991; Le Floch *et al.*, 2005).

Seed treatment with fungi such as *Trichoderma harzianum* Rifai and bacteria such as *Bacillus subtilis* under controlled environmental conditions decreases severity of root rot and increases stand as well as plant height compared to untreated seeds in pathogen-infested soil (Mao *et al.*, 1999). According to Galland and Paul (2001), *Serratia plymuthica* (Dyar) Bergey strain (B-781) was observed to control *P. perplexum* *in vitro*.

Extensive greenhouse and field trials were conducted during the past several years to identify the most effective fungicide treatments for *P. ultimum*. Results have demonstrated the need to use a highly effective fungicide against *P. ultimum* (Paultiz *et al.*, 1992).

2.2.3 *Rhizoctonia solani* Kühn

2.2.3.1 Introduction

Rhizoctonia solani Kühn is a common soil-borne fungus with a high competitive saprophytic ability (Yulianti *et al.*, 2006). The pathogen *R. solani*, which occurs in field, vegetable, ornamental, nursery and greenhouse crops is well known worldwide, including in South Africa (Csinos and Stephenson, 1999; Lewis and Lumsden, 2001).

This pathogen causes large economic losses to growers due to diseases such as damping-off, pre-emergence sprout stem cankers, fruit decay and foliage diseases on a wide host range (Parmeter, 1970; Herr, 1995; Lahkim *et al.*, 2000). The diseases are generally more severe in sandy soils where rainfall is low (Gill *et al.*, 2001). The pathogen is found worldwide in all natural soils and can survive indefinitely in warm to hot temperatures and moderate moisture levels (Darrance *et al.*, 2004; Patrício *et al.*, 2006). Rhizoctonia diseases are difficult to control because this pathogen has a wide host range and survives as mycelium in organic matter or as sclerotia in soil (Ross *et al.*, 1998). There are already some fungicide combinations available for controlling damping-off caused by *R. solani*. Even so, the diseases continue to be a major problem and there is a need to evaluate alternative fungicides (Lisker and Meiri, 1992).

2.2.3.2 Origin taxonomy and distribution

The taxonomy and nomenclature of *R. solani* has been a source of confusion and controversy for many years (Parmeter, 1970). Kühn (1858) described the fungus for the first time on a diseased potato tuber and named it *Rhizoctonia solani*. Since then, this fungus has gained the reputation of being a serious pathogen (Parmeter, 1970). The genus *Rhizoctonia* belongs to the class Basidiomycetes, family Thelephoraceae (Ceresini, 1999). *R. solani* is very common in moist soils and can survive on a wide range of host plants (Nuez, 2001).

According to Camporota and Perrin (1998), *Rhizoctonia* species can be divided into three groups based on the nuclear condition of vegetative cells: uni, bi and multinuclear, and can be divided into several anastomosis groups characterized by independent genetic units that often show different host ranges. *R. solani* has different anastomosis group and is a diverse and destructive pathogen of vegetables, legumes, ornamentals, and trees throughout the world (Ross *et al.*, 1998, Leammlen, 2004). *R. solani* is a sub-group that differs from other *Rhizoctonia* spp, in many physical and behavioural characteristics, including temperature preferences, species and type of plants attacked and region of occurrence (Parmeter, 1970).

2.2.3.3 Morphology and cultural characteristics

Historically, fungi in the *R. solani* complex have been characterized and identified based on the concept of hyphal anastomosis and morphological characteristics. *R. solani* exhibits major

variations in cultural morphology and in pathogenicity. The sclerotia are usually flattened and elongated rather than spherical (Agrios, 2005). On cultural media, sclerotia from different isolates vary in number, shape and size (Naiki and Ui, 1978).

The hyphae of *R. solani* have many nuclei, commonly four to eight per cell. This distinguishes it from similar fungi that have only two nuclei per cell. Furthermore, *R. solani* has the following characteristics: special type of cross wall within the hypae, called a dolipore septum, each cell is multinucleate; the branches are produced at right angles (Parmeter, 1970, Laroche *et al.*, 1992). Gill *et al.* (2001) demonstrated that *Rhizoctonia* hyphae could survive *in vitro* at moisture levels below the permanent wilting point.

2.2.3.4 Epidemiology and ecology

Rhizoctonia solani occurs anywhere in a field, but is usually more prevalent in lettuce in fields with high green organic matter with poor drainage or compacted soil (Rudd *et al.*, 1987). According to Gill *et al.* (2001), *R. solani* incidence appears to be sporadic and inconclusive. *R. solani* can affect plant seeds, bulbs or cuttings in the nursery, greenhouse or field and can also rapidly colonize pasteurised soil as well as soilless mixes from contaminated container equipment, planting materials and dust (Mercier and Manker, 2005).

Lettuce damping-off may occur during two phases of the development of the plant, namely shortly after seedlings germinate, but before they emerge, and after seedling emergence (Rudd *et al.*, 1987). *R. solani* can survive as mycelium or sclerotia in the soil for a long period of time in the absence of host plants (Chung *et al.*, 2005).

2.2.3.5 Symptoms

Infection of roots or foliage by *R. solani* depends on the ability of fungal hyphae to spread several millimetres through the soil (Otten *et al.*, 2004). Lettuce heads affected by bottom rot show lesions in the lower leaves as the disease progresses. Lettuce drop is associated with an aqueous rot and wilting of the heads that can depreciate their commercial value (Patricio *et al.*, 2006).

Since the pathogen has a propensity for attacking at any growth stage it can cause different symptoms on the same host depending on the time of infection (Lewis and Lumsden, 2001).

The typical disease symptoms include severe foliar browning from the base of the plant upwards and, in some cases, root and stem-base rots (Litterick and McQuilken, 1997).

Rhizoctonia solani is most active at temperatures of 15°C-18°C, when the soil is moderately moist. Dry or waterlogged soil tends to inhibit fungal development. The presence of fresh green manure might suppress the saprophytic growth of *R. solani* in these soils (Jones, 1997; Csinos and Stephenson, 1999; Yulianti *et al.*, 2006). With plants such as lettuce and cabbage, the lower leaves that touch the ground or are close to it are attacked at the petioles and midribs, on which the fungi produces reddish-brown, slightly sunken lesions, while the entire leaf becomes dark and brown (Agrios, 2005). As the disease progresses, the fungus grows from leaf to leaf inside the head of lettuce and near the stem. The outer leaves of lettuce wilt and become yellow (Rudd *et al.*, 1987).

Rhizoctonia solani can attack vegetables including lettuce and potatoes at all stages of growth. Several types of symptoms may be observed; on young sprouts when the conditions favour the fungus, the sprout may be killed before it emerges (Weinhold *et al.*, 1982). The fungus can attack potato stems, stolons and roots. Dark brown lesions or cankers are produced on the lower section of developing stems (Brewer and Larkin, 2005).

The pathogen is able to induce post-emergence damping-off and can, after transplanting to pathogen-infested soil, cause root rot, foliar blight or bottom rot. *R. solani* may cause seed and seedling diseases on greenhouse-transplanted lettuce (MacNab, 2004). Disease severity on potato is associated with cankers on under-ground stem parts and a diminished root system (Lahkim *et al.*, 2000). Lucas (1975), cited by Csinos and Stephenson (1999), reported *R. solani* AG-4 to be responsible for sore shin symptoms on tobacco leaves. *R. solani* AG-3 causes small water-soaked lesions on seedlings and can develop into large circular spots with concentric rings in field tobacco (Csinos and Stephenson, 1994). The dark brown lesions are often found on roots and stolons, and in severe cases the cankers girdle the stems, which may eventually collapse and die. On growing potato resetting and inward rolling of the upper leaves are observed (Weinhold *et al.*, 1982).

2.2.3.6 Control of *Rhizoctonia solani*

With *R. solani*, as with other organisms, successful control measures are determined by the characteristics of the pathogen, the host crop and the environment (Leach and Garber, 1970).

The control of diseases caused by *R. solani* is more efficient when integrated measures are adopted, including treatment of seeds with recommended fungicides, use of seeds with good sanitary and physiological quality, and certain cultural practices (Meyer *et al.*, 2005).

Sanitation and the use of clean growing media and plant material is the primary way of preventing damping-off and other root diseases (Mercier and Manker, 2005). However, it is obvious that several methods are available for controlling *R. solani* infection. The selection of materials and methods used will depend on the degree of protection required and on the cost that can be reasonably justified (Ceresini, 1999). Moreover the recommended methods for control of *R. solani* include the use of resistant cultivars, low nitrogen fertilization, limitation of long periods of leaf wetness, shade, poor drainage, and late afternoon and evening irrigation (Green *et al.*, 1999).

Crop rotation is an agricultural management tool with ancient origin; its benefits include maintenance of soil structure and organic matter. However, one of the most significant benefits of crop rotation is a small reduction in plant diseases caused by *R. solani* (Peters *et al.*, 2003). Furthermore, methods that minimize prolonged contact of the plant or tubers with the pathogen, such as planting in warmer drier conditions and delay in planting until conditions favour rapid growth of crop, often allows the plant to escape infection (Brewer and Larkin, 2005).

Biological control has been used successfully to suppress both pre- and post-emergence damping-off of vegetable seedlings and transplants caused by *R. solani* (Ross *et al.*, 1998). Rhizoctonia diseases remain a persistent problem, but biological control may be an effective means of control in many instances where chemical control is not available or practical (Brewer and Larkin; 2005). Moreover, Yulianti *et al.* (2006) reported that when a higher concentration of fresh green manure amendments (10%) was used, it inhibited the severity of *R. solani*, whereas a low concentration (1%) stimulated *R. solani* attack.

Trillas *et al.* (2006) reported that several soil-borne plant pathogens including *R. solani* have been reduced by using composts made of different raw materials. However, the ability of certain composts to suppress *R. solani* may be due to the presence and activity of specific antagonists such as *Bacillus subtilis* (Montealegre *et al.*, 2003). According to Harris *et al.* (1994), damping-off is reduced in many nurseries by pasteurisation or fumigation of potting media and pots, combined with good nursery hygiene. In the field, maintaining good growing conditions and preventing injury, especially by nematodes reduces root and foot rot caused by *R. solani* (Jones, 1997).

2.3 Fungicides

2.3.1 Introduction

Efforts to manage soilborne diseases through the development of resistant cultivars have had only limited success (Reid *et al.*, 2002). Moreover the efficacy of disease control of many biocontrol organisms is not always consistent (Brewer and Larkin, 2005). Chemical control remains the main measure to reduce soilborne diseases in various vegetables and fruits (Allen *et al.*, 2004). Although the majority of fungicides evaluated have not provided adequate disease suppression (Reid *et al.*, 2002), the fungicides metalaxyl, fludioxonil and mefenoxam were shown to be effective in controlling *F. solani*, *P. ultimum* and *R. solani* (Monkiedje *et al.*, 2002; Taylor *et al.*, 2004).

2.3.2 Mefenoxam (Subdue®)

Mefenoxam (Subdue®) is an acylanilide fungicide with residual and systemic activity (Demanou *et al.*, 2006). According to Malvic and Grunden (2004), mefenoxam reduced the mycelial growth of *Phytophthora* spp. The compound is widely used to control foliar or soilborne fungal pathogens such as late blight, downy mildew, damping-off and stem and fruit rots of many plants (Pai *et al.*, 2001). According to Taylor *et al.* (2004), mefenoxam still provides reliable control of water rot diseases. Benigni and Bompeix (2006) confirmed that since the 1990s, the manufacturer Syngenta (formerly Novartis) has replaced metalaxyl with mefenoxam in all formulations; the fungicide is registered for control of oomycetes.

Mefenoxam is also called R-metalaxyl and has been on the market since 1996 under various formulations and trade names, including Ridomil Gold®, Fonganiil® Gold, Apron XL®,

Subdue[®] and Maxx[®] (Monkiedje *et al.*, 2002). It has been confirmed by Monkiedje *et al.* (2002) that mefenoxam is the main fungicide used to manage diseases caused by several oomycetes, in most countries. Mefenoxam can be used alone or in combination with other fungicides such as copper, and is reported to be the primary fungicide used to manage diseases caused by several oomycetes (Demanou *et al.*, 2006). Mefenoxam has the same activity when applied at half the rate of metalaxyl against diseases caused by different *Pythium* spp. and *Phytophthora* spp. (Benigni and Bompeix, 2006). Fravel *et al.* (2005) reported mefenoxam significantly reduced *R. solani* on rosemary and reduced disease incidence of *Fusarium oxysporum* on tomato. According to Taylor *et al.* (2004), mefenoxam was found to be the only fungicide to control pink rot and leak of potato.

2.3.3 Metalaxyl (Apron[®])

Metalaxyl (Apron[®]) is a member of the acylalanine group of fungicides used worldwide specifically to control diseases caused by oomycetes (Fisher and Hayers, 1984). The compound was introduced in 1977 and is a systemic fungicide which is highly active against the Peronosporales. Metalaxyl is effective against soilborne and foliar diseases (Urech *et al.*, 1977). Moonkiedje *et al.* (2002); (2007) reported that due to its broad-spectrum activity, metalaxyl is registered for use on a wide range of crops and in several countries. The compound is taken up by roots, leaves, green stems and shoots and transported acropetally within the plant and inhibits fungal protein synthesis (Urech *et al.*, 1977; Buchenauer, 1990 cited by Moonkiedje and Spiteller 2002).

In South Africa metalaxyl was introduced into tobacco cultivation during 1978, in the Northern Province (van Jaarsveld and Drenth, 2002). Utkhede and Smith (1991) reported that metalaxyl can suppress the pathogen *P. ultimum* without eradicating it, and this chemical can be used as a preventative rather than curative treatment in nurseries.

Metalaxyl is fungistatic to *P. cactorum* and *P. cambivora*, but fungicidal to *P. ultimum* (Utkhede and Smith, 1991). According to Bhat *et al.* (1993) metalaxyl protects tobacco seedlings by inhibiting the growth of *Phytophthora nicotianae* Brenda de Haan mycelium and sporangia both *in vivo* and *in vitro*. In South Australia, metalaxyl at the rate of 0.25 g a.i.l⁻¹ has been widely used and effective on *Phytophthora*, and *Pythium* spp. diseases of lettuce since its release in 1979 (Wicks and Wolfe 1998). According to Morgan (1984), metalaxyl

can completely control *Bremia lactucae* Regel for 20 weeks after its application. Utkhede (1987) reported similar results for the ability of metalaxyl to reduce the diseases caused by *F. solani* and *P. ultimum*.

Falloon *et al.* (2000) reported that in New Zealand metalaxyl seed treatment used alone failed to control downy mildew caused by *Peronospora viciae* (Berk) Gaumon in pea crops but when combined with fludioxonil gave complete control.

A combination of metalaxyl and thiabendazol controled root rot of apple seedlings caused by all root pathogens including *P. ultimum* under greenhouse conditions. Furthermore, metalaxyl was found to be effective in inhibiting the growth of species of *Fusarium*, which were non-pathogenic to apple seedlings (Utkhede and Smith, 1991). According to Benson (1992) and Monkiedje *et al.* (2007), metalaxyl can be used to control root and crown rots in ornamental production.

2.3.4 Fludioxonil (Celest[®] XL)

Fludioxonil (Celest[®]) belongs to the chemical class of phenylpyrroles, and it constitutes a new class of non-systemic and protective fungicides (Rosslenbroich and Stuebler, 2000; Errampalli, 2004). Fludioxonil has a worldwide registration on major food crops such as cereals, corn, sorghum, potato and legume vegetables (Zang *et al.*, 2001). According to Zhang and Timmer (2007) fludioxonil could be the new alternative fungicide to thiabendazole (TBZ) and imazalil for diplodia stem-end rot control during fruit drenching treatment. Fludioxonil has been shown to exhibit broad-spectrum activity against plant pathogenic fungi (Errampalli, 2004). Fludioxonil is the active ingredient in registered products used as seed treatments for many crops, furthermore fludioxonil can be used as a foliar spray treatment and has been approved as a post-harvest treatment on fruit such as apricot, peaches and plums. According to Zang *et al.* (2001), fludioxonil can be used for protection against seedborne and soilborne fungi that cause seed decay, damping-off and seedling blight, such as *Fusarium* spp. and *Rhizoctonia* spp..

Elmer and McGovern (2004) reported that fludioxonil is the most effective product available to control fusarium wilt of cyclamen. The authors further said that fludioxonil provides the best protection against fusarium wilt when compared to other chemicals such as thiophanate

methyl, azoxystrobin and the benzimidazoles group. However, Elmer and McGovern (2004) found that fludioxonil is the most effective product available, but it has poor curative properties against *Fusarium* spp. Reid *et al.* (2002) reported that the fungicide fludioxonil may limit plant death caused by *Fusarium* spp.. Noguchi *et al.* (2007) found that fludioxonil has a strong antifungal activity against filamentous fungi such as *Botrytis cinerea* Pers.:Fr., *Alternaria brassicicola* Legent, *Alternaria alternata* (Fries) Keissler and *Colletotrichum lagenarium* (Pass.) Ellis et Halsted. Rosslendroich and Stuebler (2000) similarly found that fludioxonil could inhibit spore germination, germ-tube elongation and mycelium growth of *B. cinerea*.

2.4 Conclusion

Lettuce is a popular leaf vegetable eaten all year round because it is cultivated both outside and in the greenhouse (de Vries 1997). Wilks and Wolfe (1998) reported that lettuce is a high-value crop and relatively susceptible to fungal damage. Therefore control strategies should be effective in the long run.

Diseases caused by *Fusarium solani* are a limiting factor in plant production, and they are one of the main causes of the reduction of yields. *F. solani* causes the death of young and adult plants, with consequent economic losses (Rojo *et al.*, 2007). Many *F. solani* diseases however are ameliorated by cultural and cropping practices. On some hosts the diseases are treatable with a few fungicides. Most biological control methods fail to give consistent results (Herr, 1995). Alternative methods to protect plants from *F. solani* diseases are needed.

Globally agricultural production is lost each year due to various pests and diseases (Champagain *et al.*, 2007). Soilborne pathogenic microorganisms affecting plant health are the main and constant menace to food production worldwide (Compant *et al.*, 2005). *Pythium* spp. have a wide plant host range, allowing them to persist in the soil under a variety of crop rotation conditions (Agrios, 2005). Therefore an effective treatment for control of damping-off caused by *Pythium* spp. on lettuce seedlings is needed.

Rhizoctonia solani is a pathogen with a high competitive saprophytic ability. The fungus is one of the important causal agents affecting lettuce heads, causing bottom rot, lettuce drop and damping-off (Patricio *et al.*, 2005). *R. solani* can attack over 500 host species (Grosch *et*

al., 2005). Over the last 15 years, the significance of *R. solani* has increased worldwide. The pathogen causes important diseases such as black scurf on potato, late sugar beet rot, bottom rot on lettuce and damping-off diseases on various vegetable crops (Grosch *et al.*, 2006).

Rhizoctonia solani on lettuce is particularly difficult to control because resistant varieties are not available and few chemicals are registered against this pathogen (Duffy, 2000). Additionally some fungicides vary in effectiveness among and within anastomosis groups (AG) of *R. solani*. The control of diseases is efficient when integrated measures are adopted and should start early during land preparation (Meyer *et al.*, 2005). There is still considerable interest in finding alternative fungicides for suppression of *R. solani* on lettuce seedlings.

The control of the soilborne pathogens *F. solani*, *R. solani* and *P. ultimum*, which cause seed rots, pre- and post-emergence root and stem diseases of young seedlings is difficult because of their ecological behaviour, their extremely broad host range and the high survival rate of resistant forms such as chlamydospores and sclerotia under different environmental conditions (Grosch *et al.*, 2005).

However, chemical fumigants and seed treatments can provide some control, but they are not always practical or effective, and integrated, sustainable disease control options are desirable. In the present study the efficacy of the fungicides fludioxonil (Celest[®]), metalaxyl (Apron[®]) and mefenoxam (Subdue[®]) was evaluated for eventual incorporation into an integrated control programme on lettuce seedlings.

CHAPTER 3: MATERIALS AND METHODS

3.1 Lettuce seed and seedlings

Lettuce (cultivar Green Oak) seeds used in this experiment were supplied by Syngenta South Africa (Pty) Ltd. Lettuce seeds were planted by hand at 1.0 cm deep in seedling trays; 3-week old lettuce seedlings (cultivar Green Oak) were obtained from the University of Pretoria green house (UPGH) and transplanted into pots filled with pasteurised growing medium

3.2 Pathogens

Cultures of *Pythium ultimum* (UPGH024), *Rhizoctonia solani* (UPGH122) and *Fusarium solani* (UPGH122) were obtained from the culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria. Cultures were maintained on potato dextrose agar (PDA) (Merck, Johannesburg) at 20°C with a 12 h- light/dark regime for use as inoculum.

3.3 Fungicides

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

3.4 *In vitro* culture trials

Autoclaved PDA was augmented with the following fungicides at the rate of: metalaxyl (Apron[®]) 0.21 ml/L medium, fludioxonil (Celest[®]) 0.25 ml/L medium, mefenoxam (Subdue[®]) 0.27 ml/L medium. The media was then poured into Petri dishes (90 mm diameter).

Discs (6 mm diameter) cut from the actively growing regions of four-day old *Pythium*, *R. solani* and *F. solani* cultures respectively using a cork borer were placed in the centre of each Petri dish. There were three replicates of nine Petri dishes per treatment for each pathogen. Petri dishes were sealed with Parafilm[®] and incubated under fluorescent light at 25°C for nine

days. The diameter of colony growth per Petri dish was recorded in millimetres on the ninth day after inoculation. The experiment was repeated three times.

3.5 *In vivo* greenhouse trials

3.5.1 Infection studies

In Experiment 1, pasteurised lawn dressing growing medium (Braaks, Pretoria) was used to fill 128-cell polystyrene seedling trays. Two seeds (later thinned to one) were planted at a depth of one centimetre in each cell. Appropriate controls in infested and uninfected growing medium were also set up. There were four replications of 50 seeds per treatment (two different treatments per tray). In Experiment 2, pots (7 cm x 12 cm) were filled with pasteurised lawndressing growing medium (Braaks, Pretoria) and 3-week old seedlings were transplanted in each pot. The inoculations were done artificially, four mycelial discs (4 mm diameter) 7-day old were placed close to lettuce seedlings root during the transplantation. There were three replications of six pots containing three plants each. Seedling trays and pots were placed in a randomised block design in a controlled environment room at 20-26°C with a 12h-light/dark regime. The position of the seedling trays and pots was changed daily in the room. Seedling trays and pots were watered daily to maintain field capacity, except on the day of chemical application for avoiding the leaching of chemicals.

3.6 Fungicide treatments

Two concentrations (single and double dose) of three fungicides namely: fludioxonil, mefenoxam and metalaxyl were applied as rescue treatments of lettuce seedlings. The same rate of each of the above fungicides was applied in Experiment 1 and 2.

All fungicides were applied to the growing medium as drench treatments at the recommended concentration as described in Table 3.1. Fungicides were sprayed three times before harvesting using a hand-held sprayer (knapsack sprayer) on the 1st day during seedling transplantation and on the 14th and 28th days after transplanting on the day. The control was sprayed with tap water. Seedlings were sprayed until run-off, to make sure that the growing medium was drenched with fungicide.

Table 3.1: Active ingredient, product name, active median and milliliter of product mixed in 1.5 liter of water

Active ingredient*	Products	Active median (gai/l)	ml of product 1.5l mixed/m ²
Metalaxyl (1X)	Apron [®] XL ES	350	0.53
Metalaxyl (2X)	Apron [®] XL ES	350	1.06
Fludioxonil (1X)	Celest [®] XL	240	0.74
Fludioxonil (2X)	Celest [®] XL	240	1.54
Mefenoxam (1X)	Subdue [®] MAXX	100	0.67
Mefenoxam (2X)	Subdue [®] MAXX	100	1.34

*(1X) = Single dose; (2X) = Double dose.

3.7 Greenhouse evaluation

Percentage seedling emergence and percentage diseased plants relative to the controls were recorded on the 30th day after planting. Emerged seedlings were counted per replication per treatment and the average was calculated. Phytotoxicity was measured as growth (dry mass) and any burning, chlorosis or necrosis was recorded. Percentage abnormal seedlings was determined.

During harvest, roots were washed with tap water and disease symptoms on the leaves, stems and roots were evaluated. Roots were then excised from the shoots with scissors, placed into brown paper bags (28 cm x 15 cm) dried for 48 h into the oven at 65°C. The dry mass of root and shoots was recorded.

3.8 Statistical methods

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* trial

Results from the *in vitro* study indicate the fungicides metalaxyl, fludioxonil and mefenoxam were effective in reducing mycelial growth of *Fusarium solani* on the ninth day, with metalaxyl and fludioxonil giving the best results (Table 4.1). These results indicate that only fludioxonil was able to significantly reduce *R. solani* mycelia growth on the ninth day; the effect of metalaxyl was very low and mefenoxam was ineffective in reducing *R. solani* mycelial growth when compared to the untreated control (Table 4.1). In contrast, no growth was recorded with mefenoxam and metalaxyl at both single and double dosages against *Pythium ultimum* mycelia *in vitro* on the ninth day when compared to the untreated control. The same trend was observed with fludioxonil (Table 4.1).

Table 4.1: Effect of metalaxyl, fludioxonil and mefenoxam on *Fusarium solani*, *Rhizoctonia solani* and *Pythium ultimum* mycelial growth *in vitro* and the percentage of inhibition on the ninth day after inoculation

Treatment*	Pathogen					
	<i>Fusarium solani</i>		<i>Rhizoctonia solani</i>		<i>Pythium ultimum</i>	
	Colony diameter (mm)	Inhibition (%)	Colony diameter (mm)	Inhibition (%)	Colony diameter (mm)	Inhibition (%)
Control (Uninoculated)	90.00d**	0	90.00a	0	90.00a	0
Metalaxyl (1X)	47.00b	48	64.00b	29	0.00c	100
Metalaxyl (2X)	42.33a	54	62.50b	32	0.00c	100
Fludioxonil (1X)	42.00a	54	1.50c	96	2.00b	78
Fludioxonil (2X)	40.33a	56	1.50c	96	1.16c	83
Mefenoxam (1X)	60.83c	34	90.00a	0	0.00c	100
Mefenoxam (2X)	63.11c	30	90.00a	0	0.00c	100

* (1X) = Single dose; (2X) = Double dose; **Values in column per pathogen followed by different letters indicate difference is significant ($P = 0.05$).

4.2 *In vivo* trial in the greenhouse

4.2.1 *Fusarium solani*

Experiment 1: *Fusarium solani* had little effect on the emergence of lettuce seedlings (Table 4.2). However there was a definite trend in the control of this pathogen on lettuce using the two fludioxonil treatments, at 1X and 2X recommended dosage. The percentage of diseased seedlings was lower than the untreated inoculated control but not significantly. However, there were fewer abnormal seedlings in the untreated control. The dry mass of the shoots in the fludioxonil treatments was significantly higher than that of both controls and all other treatments. The same trend was evident in the root mass although there were no significant differences among treatments (Table 4.2). The metalaxyl 1X and mefenoxam 2X treatments had significantly more abnormal seedlings than the uninoculated control.

Experiment 2: As seedlings were 3-weeks old at transplanting, emergence was not measured but only survival of seedlings. *Fusarium* significantly reduced the survival rate of the inoculated untreated control, while all treatments except mefenoxam 2X had 100% survival rates. The two fludioxonil treatments 1X and 2X, as in Experiment 1, were most effective in controlling the disease and showed 0% diseased seedlings. The other fungicides were also effective when compared to the inoculated control. Metalaxyl 1X and 2X and mefenoxam 1X resulted in more abnormal seedlings than the untreated, inoculated control. The mefenoxam 2X however had significantly fewer abnormal seedlings. Only fludioxonil treatments had a significantly higher dry shoot mass than the inoculated untreated control whilst mefenoxam 1X and 2X treatments had a significantly lower shoot mass than the inoculated untreated control. There were no significant differences in dry mass among the treatments compared with the inoculated, untreated control (Table 4.2).

Table 4.2: Effect of lettuce seedling treatment with metalaxyl, mefenoxam and fludioxonil at single or double dosages against *Fusarium solani* in greenhouse experiments

Treatment*	Emergence/survival of seedlings (%)		Diseased seedlings (%)		Abnormal seedlings (%)		Dry mass shoots (mg)		Dry mass roots (mg)	
	Exp1**	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Control (Inoculated)	99b	94.4a	9ab	59.3e	16.5bc	22.23b	2.47a	1.87b	1.76a	0.83abc
Control (Uninoculated)	97.5ab	100b	0.5a	0a	4ab	0a	2.45a	2.83d	1.73a	1.1c
Metalaxyl (1X)	97.5ab	100b	14bc	53.7de	18.5c	46.3cd	2.97a	2.0bc	2.02a	0.8abc
Metalaxyl (2X)	97ab	100b	21.5c	48.17d	15bc	51.9d	2.42a	1.87b	1.69a	0.7ab
Fludioxonil (1X)	98ab	100b	3ab	0a	8abc	0a	5.76b	2.43cd	2.69a	0.9abc
Fludioxonil (2X)	95ab	100b	2a	0a	1a	0a	7.16b	2.37bcd	2.46a	1.03bc
Mefenoxam (1X)	96.5ab	100b	14bc	16.7b	16.5bc	37c	2.04a	1.23a	1.61a	0.83abc
Mefenoxam (2X)	94a	98.1ab	21.5c	31.47c	18c	3.7a	2.36a	1.2a	2.03a	0.67a

*(1X) = Single dose; (2X) = Double dose. **Exp1= Experiment 1 Exp2 = Experiment 2. Values in column followed by same letter are not significantly different ($P = 0.05$).

4.2.1.1 Symptoms

During the trial severe necrotic symptoms of *F. solani* were observed on seedling roots due to infection of the inoculated untreated control plants seemingly retarding the growth of the lettuce seedlings. *F. solani* caused root rot and reddish-brown lesions on the stem below and above the soil line (Figure 4.1).

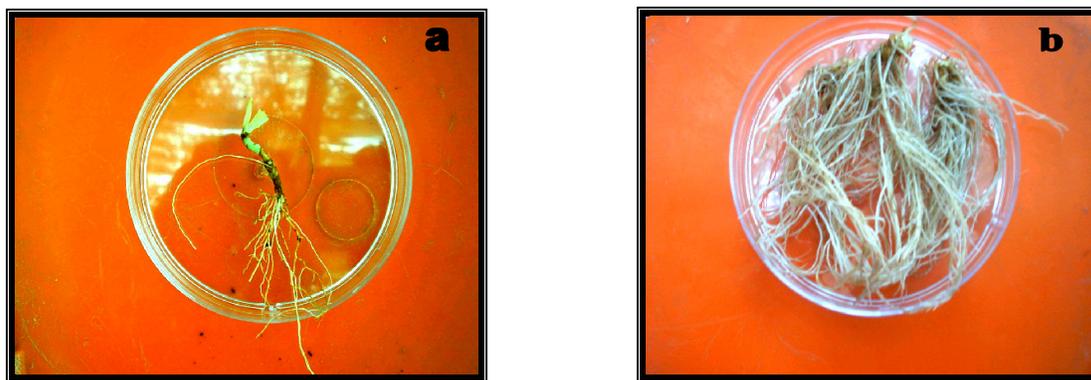


Figure 4.1: (a) Disease symptoms caused by *Fusarium solani* on a lettuce seedling stem and roots in the untreated, inoculated control and (b) roots of uninoculated lettuce seedlings.

4.2.2 *Pythium ultimum*

Experiment 1: *Pythium* significantly reduced emergence of lettuce seedlings in all the treatments except metalaxyl 2X and mefenoxam 1X (Table 4.3). This probably occurred as pre-emergence damping-off before the first spray treatment. However, when considering percentage diseased seedlings, only the two fludioxonil treatments controlled *Pythium*. The fludioxonil treatment also had the least abnormal seedlings. However, the fludioxonil 2X treatment had a lower dry root mass when compared to the uninoculated control. This was also found in the metalaxyl 1X and mefenoxam 2X treatments. There were no significant differences in dry shoot mass among all treatments and controls (Table 4.3).

Experiment 2: Although the untreated inoculated control had a 100 % survival rate, it had by far the highest percentage of diseased seedlings (63 %). Mefenoxam 1X and 2X treatments had the lowest survival rate. All the treatments significantly controlled the disease with fludioxonil 1X and 2X and mefenoxam 2X treatments being most effective. Metalaxyl 1X and mefenoxam 1X and 2X treatments had significantly more abnormal seedlings than the uninoculated control, whilst the mefenoxam 1X treatment had more abnormal seedlings than the untreated, inoculated control. The dry shoot mass of all the treatments except fludioxonil 1X and 2X did not differ significantly from the uninoculated control whilst all the other treatments had lower dry root masses (Table 4.3).

Table 4.3: Effect of lettuce seedling treatment with metalaxyl, mefenoxam and fludioxonil at single or double dosages against *Pythium ultimum* in the greenhouse experiments.

Treatment*	Emergence/ survival of seedlings (%)		Diseased seedlings (%)		Abnormal seedlings (%)		Dry mass shoots (mg)		Dry mass Roots (mg)	
	Exp1**	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Control. (Inoculated)	78ab	100b	13b	63e	26.5d	20.4c	1.15a	0.7a	0.62ab	0.38a
Control (Uninoculated)	94.5b	100b	3a	0a	6.5abc	0a	1.65a	2.57de	0.92b	1.33d
Metalaxyl 1X	60a	98.1b	9.5ab	13cd	3.5ab	11.1b	1.2a	1.83c	0.55a	0.63bc
Metalaxyl 2X	76ab	100b	10.5b	13cd	13bc	7.ab	1.21a	1.27b	0.63ab	0.47abc
Fludioxonil 1X	54.5a	100b	3.5a	0a	2.5a	0a	1.38a	2.83e	0.62ab	1.57d
Fludioxonil 2X	53.5a	100b	3.5a	3.7ab	3.5ab	0a	0.99a	2.2cd	0.41a	0.63bc
Mefenoxam 1X	73.5ab	81.5a	9.5ab	9.27bc	9abc	27.8c	1.26a	1.27b	0.70ab	0.63bc
Mefenoxam 2X	60a	81.5a	14.5b	3.7ab	14c	9.27b	0.73a	0.93ab	0.34a	0.43ab

*(1X) = Single dose; (2X) = Double dose. **Exp1= Experiment 1 Exp2 = Experiment 2. Values in column followed by same letter are not significantly different ($P=0.05$).

4.2.2.1 Symptoms

During the harvesting the basal part of the stem of some lettuce seedlings grown in media inoculated with *P. ultimum* was soft and thin. The plants showed symptoms of root rot and stunting caused by *P. ultimum* (Figure 4.2).

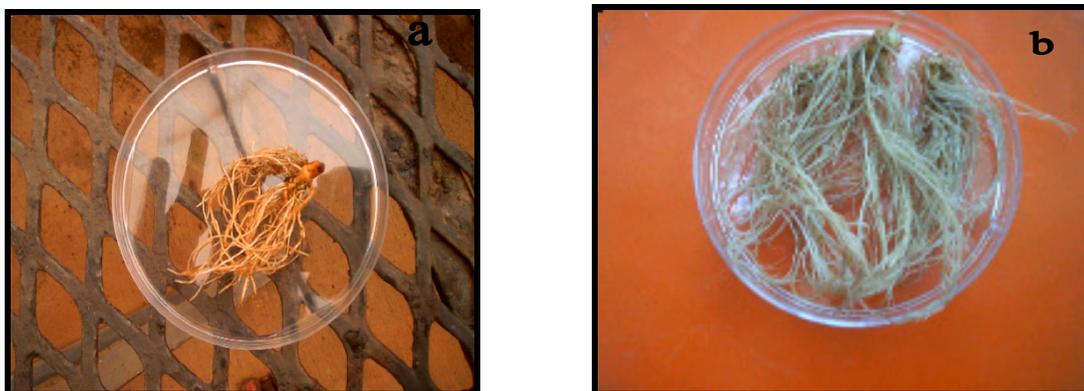


Figure 4.2: (a) Lettuce seedling roots infected with *Pythium ultimum* in the untreated control and (b) roots of uninoculated lettuce seedlings.

4.2.3 *Rhizoctonia solani*

Experiment 1: All fungicide treatments in the *Rhizoctonia*-inoculated medium showed a significantly higher percentage emergence than the untreated, inoculated control. Fludioxonil 1X and 2X treatments resulted in significantly fewer diseased and abnormal seedlings when compared to the untreated inoculated control and did not differ significantly from the uninoculated control. The same trend was evident in terms of increased dry mass of the roots and shoots particularly with fludioxonil 1X but also fludioxonil 2X treatments (Table 4.5).

Experiment 2: As in Experiment 1, all fungicide treatments resulted in a significantly higher percentage emergence than the untreated, inoculated control. Likewise, treatment with fludioxonil 1X and 2X resulted in significantly fewer diseased and abnormal seedlings when compared to the untreated inoculated control and did not differ significantly from the uninoculated control. Treatment with metalaxyl 1X and 2X resulted in significantly more abnormal seedlings than both controls. Although all the treatments had significantly higher

dry masses of shoots and roots than the untreated inoculated control, only fludioxonil 2X did not differ from the uninoculated control in dry shoot mass and mefenoxam 2X in dry root mass (Table 4.4).

Table 4.4: Effect of lettuce seedling treatment with metalaxyl, mefenoxam and fludioxonil at single or double dosages against *Rhizoctonia solani* in greenhouse experiments

Treatment*	Emergence/ survival of seedlings (%)		Diseased seedlings (%)		Abnormal seedlings (%)		Dry mass shoots (mg)		Dry mass roots (mg)	
	Exp1**	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Control(Inoculated)	92.5a	62.97a	11cd	24.1c	27.7d	24.1c	2.4ab	0.33a	1.51abc	0.17a
Control (Uninoculated)	97.5b	100d	2ab	0a	10.5ab	0a	2.35ab	2.93c	1.51abc	1.27d
Metalaxy (1X)	97b	83.3c	8bc	57.4d	22cd	42.6d	1.59a	1.73b	1.41abc	1.03cd
Metalaxy (2X)	97.5b	74.1b	17d	64.8e	22.5cd	40.7d	1.52a	1.5b	1.15abc	0.77b
Fludioxonil (1X)	99b	100d	1a	0a	6.5a	0a	3.83c	1.8b	1.73c	0.83bc
Fludioxonil (2X)	99b	100d	1.5a	0a	13.5abc	0a	3.28bc	2.7c	1.59bc	1.03cd
Mefenoxam (1X)	99b	96.3d	9.5c	22.2b	24.5cd	11.1ab	1.28a	1.83b	0.83a	0.83bc
Mefenoxam (2X)	97.5b	94.5b	12.5bc	40.5d	18.5.cd	16.7bc	1.22a	1.63b	0.95ab	1.1d

*(1X) = Single dose; (2X) = Double dose; Fludioxonil (1X) =Single dose. **Exp1= Experiment 1, Exp2 = Experiment 2.

Values in column followed by same letter are not significantly different ($P = 0.05$).

4.2.3.1 Symptoms

It was observed during harvesting that *R. solani* caused damping-off of lettuce in the inoculated, untreated control seedlings and also caused reddish-brown lesions on the stem below the soil line (Figure 4.3).

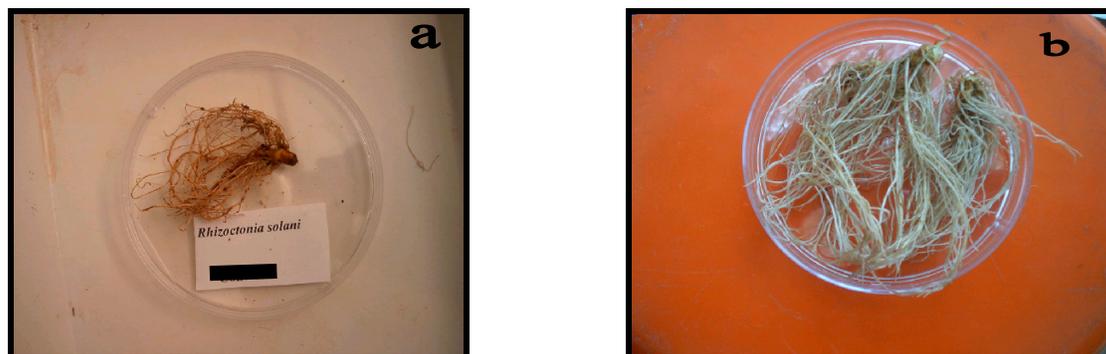


Figure 4.3: (a) Disease symptoms caused by *Rhizoctonia solani* on lettuce seedling roots in the untreated inoculated control and (b) roots of uninoculated lettuce seedlings.

As it was initially difficult to distinguish between diseased and abnormal lettuce plants we were very conservative in the reporting of diseased plants and tended to record them as abnormal seedlings in Experiment 1. The experience gained in Experiment 1 and using older seedlings resulted in greater precision in the recording of diseased and abnormal seedlings in Experiment 2. Thus, Table 4.5 gives the percentage diseased seedlings when results obtained for abnormal and diseased seedlings in Experiment 1 were pooled and re-evaluated statistically. It is evident from Table 4.5 that the fludioxonil 1X treatment successfully controlled *Rhizoctonia* and *Pythium* and the fludioxonil 2X treatment, *Fusarium*, *Rhizoctonia* and *Pythium*, and did not differ from the positive control. All the treatments controlled *Pythium* except mefenoxam 2X.

Table 4.5: Effect of metalaxyl, mefenoxam and fludioxonil treatment at single or double dosages against *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* when results obtained for abnormal and diseased seedlings in greenhouse Experiment 1 were pooled

Treatment*	Diseased Seedlings (%)		
	<i>Fusarium solani</i>	<i>Pythium ultimum</i>	<i>Rhizoctonia solani</i>
Control (Inoculated)	25.5bc**	39.5d	39b
Control (Uninoculated.)	4.5a	9.5a	12.5a
Metalaxyl (1X)	32.5c	13ab	30b
Metalaxyl (2X)	36.4c	23.5bc	39.5b
Fludioxonil (1X)	11ab	6.0a	7.5a
Fludioxonil (2X)	3a	7.0a	15a
Mefenoxam (1X)	29.5bc	18.5abc	34b
Mefenoxam (2X)	39.5c	28.5cd	31b

*(1X) = Single dose; (2X) = Double dose. **Values in column followed by same letter are not significantly different ($P = 0.05$).

In addition, our results show that, fludioxonil increased seed germination and promoted the growth of lettuce seedlings. No phytotoxicity symptoms were observed during the evaluation of metalaxyl, fludioxonil and mefenoxam on lettuce seedlings in the current study.

CHAPTER 5: GENERAL DISCUSSION

Increase of diseases in soil has always been a problem in commercial cultivation (Gül *et al.*, 2005). *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* are among the most important soilborne plant pathogens that induce damping-off, root rot, wire stem and head rot on numerous vegetables including lettuce (Nicolle *et al.*, 2003; Chung *et al.*, 2005). The most efficient and economically viable method for disease control is the use of resistant varieties (Wada, 2003). However, the development of resistant varieties takes time to achieve so until now, fungicides have been the primary means of control of these soilborne pathogens in the short term (Olufolaju, 1993).

Three fungicides were screened and evaluated for their efficacy to control seedling diseases on lettuce *in vitro* and *in vivo* in the greenhouse.

The results reported in the current study show a significant effect of metalaxyl on *P. ultimum* growth on PDA. No growth was recorded with either doses of metalaxyl (1X) and (2X) (3.4). These results confirm metalaxyl's antifungal activity as reported earlier by Babadoost and Islam (2003). Similarly, Kerkenaan and Sijpesteijn (1981) and Hrelia *et al.* (1996) demonstrated high *in vitro* activity of metalaxyl against fungal pathogens in general and more specifically to fungi belonging to the class oomycetes. The current study indicated that metalaxyl was particularly effective in controlling *P. ultimum*. The same results were previously reported by Harris and Nelson (1999). However, in the present study complete control of *F. solani*, and *R. solani* growth on PDA was not obtained at the ninth day with metalaxyl when mean values were compared with that of uninoculated control. This can be due to insensitivity of *F. solani* and *R. solani* to metalaxyl.

In the current study it was found that, in the greenhouse, significant disease control of *F. solani* using fludioxonil was recorded for both experiments when comparing the inoculated, untreated control to treated seedlings. However, the disease control levels in Experiment 2, where seedlings were grown in pots, were less than that in Experiment 1 grown in seedling trays. The large soil volume in the pots could be the cause of the lower efficacy.

Monkiedje *et al.* (2007) conducted an experiment in the field and found that metalaxyl was effective against *Phytophthora* and *Pythium* spp. Babadoost and Islam (2003) also found that metalaxyl was effective in inhibiting the germination of sporangia and mycelial growth of *P. ultimum*. In contrast, in the present study metalaxyl did not control *P. ultimum* in the greenhouse, although *in vitro* metalaxyl effectively controlled *P. ultimum*. Pedersen *et al.* (2003) found metalaxyl seed treatment was effective in reducing severity of root rot damage to corn and soybean caused by *P. ultimum*. In contrast, Falloon *et al.* (2000) found that metalaxyl does not affect other groups of oomycetes. Metalaxyl (1X) and (2X) treatment in the present study did not give complete control of *P. ultimum* (UPGH024). Idris *et al.* (2007) reported similar results on sorghum.

Suffer and Guibert (2007) revealed that the composition of the *Pythium* community is usually not homogenous. Fontem *et al.* (2005) reported that metalaxyl (Apron[®]) is used for late blight management in potato and tomato caused by the oomycete *Phytophthora infestans*. The resistance of *P. infestans* populations to metalaxyl can be associated with severe late blight epidemics, genetic diversity and pathogen resurgence. Further research is required to determine the factors influencing insensitivity of metalaxyl to other oomycete fungi.

In the present study metalaxyl was not as effective in inhibiting *R. solani* mycelial growth *in vitro* when compared to *P. ultimum*. The same trend was recorded in Experiments 1 and 2 in the greenhouse. This confirms the inefficacy of metalaxyl to control *R. solani* as reported in previous studies. Kondoh *et al.* (2001) reported that metalaxyl is active against oomycetes, but is ineffective in controlling damping-off caused by *R. solani* on tomato plants. In contrast, Monkiedje *et al.* (2007) found that metalaxyl combined with mefenoxam significantly controlled stem rot caused by *Rhizoctonia* in ornamental production.

Kondoh *et al.* (2001) reported that metalaxyl plus tolclofos-methyl or combined with flutolanil was effective in reducing *R. solani* populations on tomato. Ghate *et al.* (1991) also reported metalaxyl to be ineffective in reducing *R. solani* on cucumber.

Our observations revealed that none of the fungicide treatments caused any phytotoxicity expressed as burning, chlorosis or necrosis to lettuce seedlings in any of the pathogen inoculated soils. This is in contrast to reports by Resh (2001), cited by Mercier and Manker (2005), who reported that fungicide drenches could cause phytotoxicity or negatively affect

growth of seedlings. Meyer *et al.* (2006) also observed phytotoxicity symptoms, such as dwarfing and leaf burning in treatments with the fungicide tebuconazole to control rhizoctonia blight of soybean.

Our results show that mefenoxam suppressed the mycelial growth of *P. ultimum* *in vitro*. However, the same trend was not recorded in the greenhouse experiments, probably because the efficacy of metalaxyl declined quickly. The present study suggests that metalaxyl should be applied at a higher dose, or as a seed treatment as reported by Perderson *et al.* (2003). The abnormality of seedlings could be influenced by the increase of *P. ultimum* inoculum levels before the drench of metalaxyl. In the present study we suggested the application of metalaxyl immediately after planting. Results from this study indicate that further research is needed to address the inefficacy of metalaxyl for the control of *P. ultimum*. Benigni and Bompeix (2006) reported that mefenoxam applied at 48 g ai 100 l⁻¹ provided a high level of control of *Phytophthora cryptogea* to control dumping-off of chicory. The present study suggests the application of mefenoxam to control various oomycetes.

Results from this study showed that mefenoxam did not control *R. solani* *in vitro* or *in vivo*. Neither of the mefenoxam doses showed inhibition of *in vitro* growth of *R. solani* or *F. solani*. In contrast, however, fludioxonil treatments inhibited mycelial growth of all pathogens, *F. solani*, *P. ultimum* and *R. solani* *in vitro*.

The high percentage of abnormal seedlings recorded in Experiment 1 can be ascribed to the fact that the lettuce was planted late in the season under suboptimal conditions. In Experiment 2 in which lettuce seedlings were cultivated in pots, better plant development was observed compared to Experience 1 (Chapter 4).

Jeffer and Schnabel (2004) reported that mefenoxam is the primary fungicide used to manage diseases caused by oomycetes and frequent fungicide applications are required to control the pathogens. Fravel *et al* (2005) and Demanou *et al.* (2006) also confirmed that mefenoxam combined with copper was more efficient in overcoming fungal resistance. Results from this study showed that a combination of mefenoxam with fludioxonil or metalaxyl is required to control *R. solani* mycelial growth on lettuce seedlings.

Bucher and Pedersen (2004) reported that the combination of mefenoxam and fludioxonil effectively reduced rot root of soybean caused by *R. solani*. In previous studies, which focused on the chemical control of rhizoctonia rot root, it was found that mefenoxam alone reduced the infection caused by *R. solani* on *Catharanthus* rose seedlings (Martinez-Espinoza *et al.*, 2004). In contrast, in the present study mefenoxam did not control *Rhizoctonia* mycelial growth on PDA either at single or double dosages. More detailed studies will be required to explain the ineffectiveness of mefenoxam on *F. solani* and *R. solani*.

The results from the present study showed that, fludioxonil significantly reduced the diseases caused by the pathogens *F. solani*, *P. ultimum* and *R. solani*, but did not give complete control of all the pathogens. Bradley *et al.* (2007) reported fludioxonil (Celest[®]) is registered in France for the control of *Fusarium* spp. and it is a seed treatment fungicide used to improve the emergence of flax (*Linum usitatissimum* L.) cultivars. Elmer and McGovern (2004) also found that fludioxonil could suppress the growth of *Fusarium* spp. and the typical symptoms of fusarium wilt of cyclamen infection. Our results support this finding that fludioxonil can be used for the control of *F. solani* on lettuce seedlings.

Verdisson *et al.* (2001) previously found fludioxonil (Celest[®]) to be effective for control of *Botrytis cinerea*, the causal pathogen of grey mould of grapevines. This was also reported in studies conducted by Walter *et al.* (2005), who found that fludioxonil was capable of controlling flower and berry infection by *B. cinerea* in booyensberry. Similarly, Rosslenbroich and Stuebler (2000) reported that fludioxonil was capable of inhibiting spore germination, germ-tube elongation and mycelial growth of *B. cinerea* on grapes, vegetables and berries, respectively. Likewise, this study should be extended in a similar way to determine the efficacy of fludioxonil on the numerous fungi that cause root rot and damping-off of lettuce.

Results from the current study show that there was no difference between fludioxonil treatments on *R. solani* when compared with the uninoculated control. This confirms the finding of Ali and Archer (2003) who evaluated the efficacy of fludioxonil *in vitro* and *in vivo* and found that the mycelial growth of *R. solani* was strongly inhibited by the fungicide and blight disease of rice was reduced. Similarly, Meyer *et al.* (2006) reported similar results on soybean. In the present study the results show that the application of fludioxonil at single (1X)

and double (2X) recommended dosages controlled *F. solani* and *R. solani* on lettuce seedlings.

More experiments have been performed *in vitro* and *in vivo* to demonstrate fludioxonil activity against several species of *Fusarium*. Ayed *et al.* (2006) reported that mycelial growth of *Fusarium oxysporum* f.sp. *tuberosi*, the causal agent of potato fusarium wilt was significantly inhibited by fludioxonil. There have also been similar studies conducted by others, which produced similar results. Fludioxonil was demonstrated to be effective against blue mould of apple (Errampali, 2004), sclerotinia drop of lettuce (Martheron and Porchas, 2004) and decay caused by *Fusarium sambucinum* (Zang *et al.*, 2001). Singh and MacGovern (2004) found fludioxonil treatment reduced infection severity and disease incidence in caladium tubers. Zang *et al.* (2001) reported the reduction of *F. solani* on maize when treated with fludioxonil.

Metalaxyl seed treatment followed by foliar spray application of metalaxyl after planting is recommended for the control of *P. ultimum* in the present study. In case of lettuce seedling transplantation, roots could be treated in metalaxyl and the soil drenched with metalaxyl at transplanting followed by foliar sprays.

CHAPTER 6: CONCLUSION

In conclusion the control of *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani* which induce seed rots and pre- and post-emergence root and stem diseases of young seedlings, causing poor establishment of lettuce was assessed in this work. Three fungicides namely metalaxyl, fludioxonil and mefenoxam were screened to test their efficacy against lettuce seedling diseases *in vitro* and *in vivo* in the greenhouse.

Metalaxyl 1X and 2X dosage treatments showed high percentages of abnormal seedlings in the *Fusarium* and *Rhizoctonia* inoculation trial, but not in the *Pythium* inoculation trial indicating that it was stress by the pathogen causing abnormalities and not fungicide phytotoxicity. Likewise, mefenoxam 1X resulted in high percentages of abnormal seedlings in the *Fusarium* and *Pythium* treatments but mefenoxam at the higher 2X dose did not differ from the uninoculated control in *Fusarium* and *Rhizoctonia* inoculated plants. Abnormal seedlings were therefore not due to fungicide toxicity.

Our results support the hypothesis that all fungicides at single and double dosages exerted an inhibitory effect on mycelial growth *F. solani*, *P. ultimum* and *R. solani* *in vitro*, except mefenoxam which failed to control *R. solani* at single and double dosages *in vitro*.

In the greenhouse trial the treatment with fludioxonil (Celest[®]) at single and double dosages inhibited diseases caused by the three fungi *F. solani*, *P. ultimum* and *R. solani* on lettuce seedlings without causing any phytotoxicity. However, further studies are required to evaluate the efficacy of fludioxonil under field conditions for control of root rot caused by *F. solani*, *P. ultimum* and *R. solani*.

Based on our results, it can be recommended that fungicides should be applied immediately after planting to prevent the build-up of soilborne inoculum. Also, fungicides should be used as foliar applications on lettuce seedlings and the fungicide mixture formulations or dosages should be at the full rate as recommended.

More information is required to determine whether the combination or succession of different active ingredients such as mefenoxam plus metalaxyl or fludioxonil plus mefenoxam could

have considerable impact on reducing soilborne disease problems in the greenhouse or under field conditions.

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