A Study of *Aphanomyces euteiches* Drechs.

Root Rot of Field Pea (*Pisum sativum* L.)

in Manitoba

BY

KIRSTEN LEA SLUSARENKO

A Thesis
submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
of the degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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I dedicate this to my Mom.

FORWARD

This thesis has been prepared in the manuscript format in accordance with the Department of Plant Science guidelines. The reference style used throughout the thesis follows that of the Canadian Journal of Plant Pathology. Abridged version of one manuscript will be submitted for publication to the Canadian Journal of Plant Pathology. This paper is entitled Geographic Incidence, Pathogenicity and Host-range of *Aphanomyces euteiches* in Manitoba.

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ABSTRACT

Aphanomyces euteiches Drechs. is an important yield reducing pathogen of field pea (*Pisum sativum* L.) in Manitoba. Currently, disease control is achieved through crop rotation or avoidance of fields with high inoculum pressure. The purpose of this study was to determine *A. euteiches* incidence, pathogenicity and host-range, as well as the effect that fungicidal and biological seed treatments have on pathogen control. A survey of commercial field peas was conducted in 1999 and 2000 to determine the incidence of *A. euteiches* in Manitoba. *Aphanomyces euteiches* was isolated from 9 of 44 fields surveyed. The *Aphanomyces* isolates originated from fields within a 400 km range extending from Morden to Russell, Manitoba. Growth cabinet experiments were carried out in 2000 and 2001, to determine if certain environmental conditions and hosts were favourable to the pathogen. Using the pea variety Carneval, seven *A. euteiches* isolates (15, 22, 24, 25, 26, 27 and 41) were studied at four growing temperatures (16, 20, 24, and 28° C) and three seedling ages (1, 2, and 3 weeks). Measurement to determine the extent of disease development included disease severity and root and shoot dry weights. Disease development was favourable at all four temperature regimes tested. Seedling age had no consistent effect on disease development. Disease severity between the seven *A. euteiches* isolates were significantly different ($p=0.05$), suggesting that pathogenic variability exists between the isolates of *A. euteiches*. To determine each isolates host-range, all seven isolates were tested against four pea varieties (AC Tamor, Carneval, Marjoret, and Trapper), eight lentil varieties (CDC Glamis, CDC Robin, Crimson, Eston, French, Indianhead, Laird, and Richlea), two chickpea varieties (CDC Desiray and CDC Yuma), six bean varieties (AC Clack Diamond, CDC Pintium, AC Scarlet, Envoy,

Navigator, and Pintoba), one Alfalfa variety (OAC Minto) and one soybean variety (Alta). All seven isolates were virulent on the four pea varieties tested and avirulent on the soybean, chickpea and bean varieties tested. Lentil and alfalfa were the only hosts to with both susceptible and resistant lines to the seven isolates. Field experiments to determine the effect of seed applied fungicides and biological agents on pathogen control were conducted in 1999 and 2000 at sites in and near Morden and in Winnipeg, Manitoba. A natural source of inoculum was relied upon at each location. The thirteen fungicide and biological seed treatments tested included seven fungicides (Aliette, Apron, Crown, Ridomil, Ronilan, Thiram, and Vitaflo-280) and two biological agents (ACM941 and AR101). Three combination treatments were also tested, consisting of both a fungicide and a biological agent (ACM941+Aliette, ACM941+Apron and ACM941+Vitaflo-280). Emergence, disease severity, root and shoot dry weights and yield were measured to determine the effect each treatment had on *Aphanomyces* root rot. Emergence was significant ($p=0.05$) at each field site, although the overall effect that individual treatments had on emergence varied between sites. Average disease severity and yield ranged from 0.3 to 3.9 and 467g to 1227g between sites, respectively. These differences may have resulted from variable environmental conditions (temperature, moisture and soil type) between sites and years, and/or the relative aggressiveness of the *A. euteiches* isolates present. An increase in inoculum pressure, resulting from growing the same crop in consecutive years could also explain the increase in disease severity. These results suggest that *A. euteiches* is more extensively spread in Manitoba than previously believed and that the isolates present are pathogenically variable. The importance of crop rotation as a method of controlling this disease is also evident since

pea, lentil and alfalfa were obvious hosts and none of the thirteen seed treatments tested significantly controlled *A. euteiches* root rot of field pea.

1.0 Introduction

Dry peas (*Pisum sativum* L.) have been grown in Western Canada since farmers started plowing the prairies over 100 years ago (Pulse Canada, 2001). Field pea production slowly started to increase in 1977 and has increased since then, except for 1983, 1989, 1990 and 1996 (Miller, 1996). Factors contributing to this increase include the opening of the European feed pea market in 1985, increased emphasis on crop diversification, crop rotation, value added processing, new industries in rural areas, and sustainability of agriculture in western Canada. While focus in earlier years was on food-quality peas, now much of the crop is used for feed (Manitoba Agriculture and Food, 2003).

Global pea production increased to 11.7 million tonnes in 2000-2001 as compared to 11.3 million tonnes in 1999-2000 (Manitoba Agriculture and Food, 2001). In 2000, Western Canada's dry pea production increased to a record 2.86 million tonnes. Manitoba is the third largest pea-producing province in Canada, behind Saskatchewan and Alberta, harvesting 5.4 and 6.5 million bushels (150,000 and 200,000 acres) of field pea in 2001 and 2002, respectively (Manitoba Agriculture and Food, 2003). Increased hog production in Manitoba has provided an outlet for locally grown peas, helping to offset limited fusarium-free feed barley and wheat supplies. Over the past 80 years, the area seeded to pea in Manitoba has varied from as low as 500 acres in 1931 to as high as 260,000 acres in 1998 (Manitoba Agriculture and Food, 2003).

Root rot, caused by the oomycete *Aphanomyces euteiches* Drechs., is a serious yield-reducing disease of pea worldwide. This disease has been reported in most pea-growing areas of North America, northern Europe, Australia, New Zealand and Japan (Pfender,

1984). *Aphanomyces euteiches* is a soil-borne fungus which infects the root and epicotyl tissue of pea. Currently, there are no fungicides registered for control of this pathogen in Canada.

Distribution of this pathogen in Manitoba is relatively unknown. To date, *A. euteiches* has only been reported in the Red River Valley on faba-bean (Lamari and Bernier, 1985) and pea (Mathur *et al.*, 1998). Current management practices include avoiding fields with a high risk for disease and the use of proper management practices. These practices include the use of lengthy crop rotations (up to 10 years with a non-host crop) and highly fertile well-textured and drained soils. *Aphanomyces euteiches* possess thick-walled oospores which allow the pathogen to overwinter and survive for extended periods of time in the soil. Determining the incidence, virulence and host-range of *A. euteiches* in Manitoba would inform producers of the diseases occurrence, as well as outline crop rotations that may reduce disease development.

There is a growing concern that the lack of awareness and the absence of control measures may result in increased incidence of Aphanomyces root rot. This is particularly serious, given the pathogen's ability to survive in crop debris for a very long period of time. Host resistance would be the most effective method of control. However, incorporation of resistance into commercial varieties has yet to be accomplished. Determining an effective seed treatment to control *A. euteiches* would provide producers with an alternative means of managing this pathogen.

1.1 Objectives

The objectives of this project were to, i) determine the pathogen's distribution in Manitoba, ii) determine the virulence and host-range patterns of the *A. euteiches* isolates

obtained in Manitoba, and iii) evaluate fungicidal and biological control agents for control of *A. euteiches* in field pea (*Pisum sativum* L.).

2.0 Literature Review

2.1 The Host

2.1.1 Field Pea (*Pisum sativum* var. *arvense* L.)

Field pea (*P. sativum*) is native to Southwest Asia and one of the first crops cultivated by man. Currently field pea is produced worldwide. Canada is third in world pea production and is one of the world's largest exporters (Manitoba Agriculture and Food, 2001). Field pea made up 68.1 percent of Manitoba's special crop acreage in 1997, and 5 percent of Canada's total dry pea production in 2000.

Field pea is grown commercially in Canada as a pulse crop and is marketed for both human and animal consumption as a dry edible, processing, fresh, feed, forage, green manure or seed crop (Pfender, 1984). These crops are harvested either at the mature green or dry-seed stage (Janick, 1986). Dry edible peas constitute the bulk of world production. Field pea is used as a high-protein food source and also as a break crop because of its ability to fix atmospheric nitrogen in symbiosis with *Rhizobium leguminosarum* (Gill and Vear, 1980). Recently, field pea became an alternative to meat and meat by-product proteins in Europe, as well as in niche markets concerned with genetically modified organisms (Manitoba Agriculture and Food, 2001).

Field pea is an annual, cool-season, nitrogen-fixing legume. It is morphologically and physiologically diverse, exhibiting variations in seed size and colour, plant height and maturity, and the presence or absence of leaves.

Approximately 60 varieties of pea are listed in the Seed Manitoba 2003 - Variety Guide and Growers Directory. Varieties are categorized according to seed colour: yellow, green, green marrowfat or maple. Morphological differences such as leaf type

(normal, semileafless or tared), maturity (very early, early, medium and late), vine length (short, medium and tall) and seed size (small, medium, large, and very large) are also outlined for the different varieties.

Many diseases are responsible for production limitations in field pea. Diseases such as *Ascochyta* blight, powdery mildew, sclerotinia, seed decay, seedling blight and root rot hinder production (Pfender, 1984). Root rot pathogens include *Pythium* spp., *Fusarium oxysporum* Schlecht. f. sp. *pisi* Snyd. & Hans., *F. solani* (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans., *Ascochyta pinodella* L. K. Jones, *Rhizoctonia solani* Kühn, *and Aphanomyces euteiches* Drechs. (Tu, 1987). Jacobsen and Hopen (1981) considered *A. euteiches* to be the most important root rot pathogen.

More than 80% of the root rot losses in the United States were attributed to *A. euteiches* (Papavizas and Ayers, 1974). Reiling *et al.* (1960) found that as the degree of root rot increased, vine and pod yield decreased. Pfender and Hagedorn (1983) determined that a logarithmic relationship existed between initial inoculum level and pea yield. At low inoculum levels, small increments of inoculum resulted in relatively large changes in yield. At higher inoculum levels, increasing increments of inoculum had less effect on yield.

2.2 The Pathogen

2.2.1 Taxonomy

Aphanomyces

The genus *Aphanomyces* was established by de Bary (1860) and was included in the order Saprolegniales. *Aphanomyces* consists of 32 species differentiated by

morphologic and pathogenic characteristics (Malvick *et al.*, 1998). Seven are soilborne pathogens that cause root rot diseases (Scott, 1961; Sing and Pavgi, 1977; Ichitani *et al.*, 1986). The name "*Aphanomyces*" was derived from Greek and means "imperceptible fungus" because of the translucent aseptate appearance of the vegetative hyphae that constitutes this pathogen (Scott, 1961).

Aphanomyces euteiches Drechsler

Aphanomyces euteiches Drechs. was first described by Jones and Drechsler in 1925 and was recognized as the causal agent of common root rot of pea. *Aphanomyces euteiches* is a soil-borne organism classified in the kingdom Chromista, division Oomycota (Moore-Landecker, 1996). Root rot caused by *A. euteiches* occurs in many geographical regions including Europe, Australia, Japan, Canada, and the United States (Papavizas and Ayers, 1974). *Aphanomyces euteiches* has been the most studied of the phytopathogenic species, causing widespread and potentially significant damage to pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.), snap bean (*Phaseolus vulgaris* L.), and red clover (*Trifolium pretense* L.) (Jones and Drechsler, 1925; Scott, 1961; Papavizas and Ayers, 1974; Holub *et al.*, 1991a). This pathogen also infects a variety of legume species (Chan and Close, 1987).

Aphanomyces euteiches f. sp. *pisi*

Since *A. euteiches* was determined to be parasitic on a broad range of hosts, especially species of legumes (Papavizas and Ayers, 1974), Pfender and Hagedorn (1982) erected two *forma specialis* to distinguish between the different *A. euteiches* types:

Aphanomyces euteiches f. sp. *pisi*, the common type pathogenic on pea, and *A. euteiches* f. sp. *phaseoli*, the type specifically pathogenic to snapbean. Root rot caused by *A. euteiches* f. sp. *pisi* is a serious disease of pea (Pfender, 1984; and Papavizas and Ayers, 1974) and is the principal-limiting factor of pea production throughout the world (Jacobson and Hopen, 1981).

2.2.2 Disease Symptoms

Aphanomyces euteiches can infect throughout the growing season, attacking all underground portions of the pea plant (Jones and Drechsler, 1925). Infection generally occurs about the time plants emerge from the ground but may also occur later in the season (Pfender, 1984). The first symptoms of disease appear on the roots approximately one or two weeks after infection. Below-ground symptoms represent the best diagnosis of the disease.

Below-ground symptoms include a pale yellow discoloration of early-infected basal stem and root tissue, followed by a soft decay of the cortical tissue (Jones and Drechsler, 1925; Lamari, 1982). *Aphanomyces euteiches* infects the epicotyls of pea at the early seedling stage and the roots at any growth stage (Papavias and Ayers, 1974). Jones and Drechsler (1925) thoroughly describe disease symptoms on pea. Infected areas of the epicotyls become water-soaked and turn brown. Severely infected epicotyls become shrunken and may rot completely, resulting in the collapse and death of the seedling. If the roots become infected, light brown lesions develop, enlarge and spread into the cortex. The cortical tissue turns dark chocolate brown, softens and is easily sloughed.

Above ground symptoms of this pathogen include stunting, wilting of the lower leaves, and sudden wilting of the entire plant or overall weak adult plants (Lamari, 1982). However, these symptoms are not necessarily unique to *A. euteiches*. Plants that do survive, exhibit severely limited yields due to poor overall growth.

Microscopic identification is based on the presence of thick-walled oospores in the cortical tissues of infected plants (Mathur *et al.*, 1998; Lamari and Bernier, 1985; Jones and Drechsler, 1925).

Accurate diagnosis of *A. euteiches* root rot is often hindered because the fungus is difficult to isolate from the infected tissue (Pfender *et al.*, 1984). Oyarzum *et al.* (1993) reported that a complex of pathogens under natural conditions caused root rot of pea. This complex not only includes different species but strains with different degrees of pathogenicity. *Aphanomyces euteiches* may be obscured on isolation plates by other root-invading fungi such as *Pythium* spp., *Fusarium solani* or *Rhizoctonia solani*. Bacteria are also a problem on isolation plates.

2.2.3 Disease Cycle

The complete life cycle of *Aphanomyces euteiches* is represented in Figure 2.1. *A. euteiches* survives in the soil as thick-walled oospores, embedded in plant debris, and can persist in a dormant state for years. The persistence of oospores in the soil appears to play an important role in common root rot of pea (Temp and Hagedorn, 1967). These oospores are believed to provide the primary inoculum for new outbreaks of root rot of pea (Scharen, 1960). When conditions are favourable, the oospores germinate in the soil.

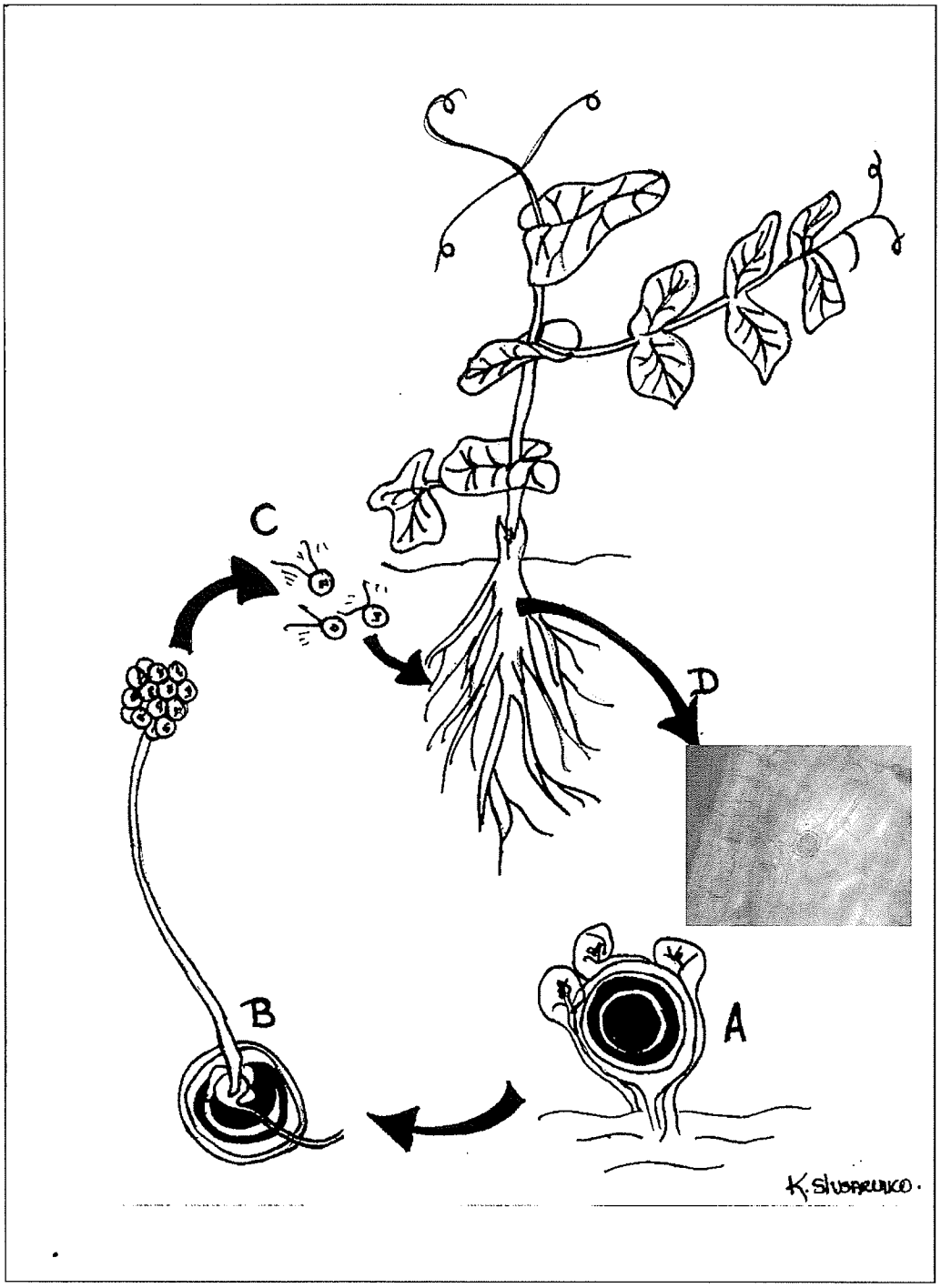

Figure 2.1. Life cycle of *Aphanomyces euteiches*. A) Resting oospore with several antheridia. B) Germinated oospore with encysted zoospores at tip. C) Biflagellate zoospores attracted to root exudates. D) Oospores within the cortical tissue of pea root.

On germination, hyphae are produced, from which asexual swimming zoospores are extruded. Following encystment, the zoospores exit the cyst and swim for a period of time. Zoospores attracted to root exudates, become static, germinate and penetrate feeder root-tips (Howard *et al.*, 1994). After infection, mycelium grows through the root cortex and forms oospores during the later stages of pathogenesis. Oospores are released into the soil as the roots decay and persist as a source of inoculum for as long as 10 years (Papavizas and Ayers, 1974; and Pfender, 1984). The primary infective agent appears to be the zoospore, since the oospores observed germinating all did so by means of zoospores (Scharen, 1960).

Jones and Linford (1925) found that the extent of infestation in a field was closely related to the number and frequency of previous pea crops. Pfender and Hagedorn (1983) reported that *Aphanomyces* could spread from an infected plant to roots of neighboring healthy plants. It is not known whether this spread occurred via mycelial growth between roots in contact with each other or by short distance zoospore movement between roots. Spread within the field was limited to a distance of five plants or approximately 18 cm from the initially infected plant. Long distance movement of the pathogen can also be achieved through infested soil or infected vines (Pfender, 1984).

2.2.4 Reproduction

Aphanomyces euteiches reproduces both sexually and asexually. The aseptate mycelium of this fungus can produce two types of spores: oospores and zoospores. Pfender (1984) reported that the thick-walled oospores, formed by sexual fusion of the oogonia and antheridia, can persist in a dormant state in the soil for several years. On

germination, the oospores form sporangia that are undifferentiated morphologically from the hyphae, which in turn produce asexual swimming spores or zoospores. *Aphanomyces euteiches* appears to be a homothallic species (Malvick and Percich, 1998a).

Optimum temperature for zoospore production was determined to be 24°C. Zoospore numbers were substantially fewer at higher and lower temperatures (Llanos and Lockwood, 1960). However, Schneider and Johnson (1952) determined the optimum range for zoospore production to be 15-20°C. Maximum mycelial growth was not necessarily associated with maximum production of zoospores. Vegetative growth was less at 20°C than at 24 or 28°C. However, as many zoospores were produced by cultures grown at 20°C than those grown at a higher temperature (Llanos and Lockwood, 1960).

Intraspecific variability has been identified in *A. euteiches*. Sexual recombination and somatic variability via asexual production of zoospores may be two sources of variability (Moore-Landecker, 1996). Hybridization and recombination may occur since antheridia have been observed to arise from different, as well as the same hypha on which oogonia are produced (Scharen, 1960). Somatic mutation or segregation, expressed as pathogenic variation among zoospores, has also been reported as a possible means by which diversity is generated (Holub *et al.*, 1991a).

2.2.5 Pathogenicity

Pathogenicity is defined as the ability of an organism to incite a disease (Poehlman and Sleper, 1995). Isolates of *A. euteiches* vary in their pathogenic ability and virulence, as measured by disease severity. Knowledge of the pathogenicity potential of

a pathogen is important for the strategy of minimizing disease occurrence and breeding for disease resistance. Holub *et al.* (1991b) observed pathogenic diversity among *A. euteiches* isolates. They also observed somatic segregation for virulence, when cultures propagated from single zoospores of a broad host-range isolate differed from the parent in pathogenicity.

Two levels of pathogenic variation among strains of *A. euteiches* have been reported. Strains causing differing levels of disease severity to different varieties of the same host (pea and alfalfa), have been referred to as physiologic specialists, races or virulence phenotypes (King and Bissonette, 1954; Beute and Lockwood, 1967; Carley, 1970; Sundheim, 1972; Manning and Menzies, 1984; Grau *et al.*, 1991). The second level of variation (pathotypes or *forma specialis*) is preferential pathogenicity to different plant species (bean, alfalfa and pea) (Pfender and Hagedorn, 1982; Grau *et al.*, 1991; Holub *et al.*, 1991b). Strains of *A. euteiches* with selective pathogenicity to bean and pea were described and had sufficient host-specificity to be recognized as *forma specialis* (f. sp.) *phaseoli* and *pisi*, respectively (Pfender and Hagedorn, 1982). However, Grau *et al.*, (1991) and Holub *et al.*, (1991b), suggest that f. sp. *pisi* is not sufficiently distinct to be a *forma specialis*. They reported that the intraspecific variation and the phenotypic and genotypic relationships among and between races, pathotypes and *forma specialis* of *A. euteiches* were poorly characterized.

2.2.6 Host-range

Host-range studies assist pathogen control by outlining suitable crop rotations. Knowing the potential host-range of the pathogen allows proper crop rotations to be

implemented, which ultimately reduces field inoculum levels and disease severity (Grau *et al.*, 1991).

Aphanomyces euteiches has been tested against several plant species since its isolation and description (Jones and Drechsler, 1925). Papaviza and Ayers (1974) compiled an extensive list of more than 80 species from 19 families reported to be hosts of *A. euteiches*. Many species are infected by *A. euteiches* (Papavizas and Ayers, 1974), including weeds and pasture legumes, which are colonized without symptoms (Chan and Close, 1987). Some plants parasitized by *A. euteiches* include alfalfa (*Medicago sativa* L.) (Delwiche *et al.*, 1987; Schmitthenner, 1964), snapbean (*Phaseolus vulgaris* L.) (Pfender and Hagedorn, 1982), faba bean (*Vicia faba*) (Lamari and Bernier, 1985) and red clover (*Trifolium pratense* L.) (E.B. Holub, unpublished data). Non-leguminous plants are not suitable hosts (Sherwood and Hagedorn, 1962).

Parasitization of legumes other than pea may also play a role in perpetuating *A. euteiches* (Sherwood and Hagedorn, 1962). Grau *et al.*, (1991) found that isolates expressed different degrees of specificity to crop species. Many species display specificity to a particular host. For example, *A. cochlioides* attacks sugarbeet and *A. astaci* attacks crayfish only (Scott, 1961). Grau *et al.* (1991) reported that isolates ranged from having multiple hosts (an isolate from pea) to being very host-specific (an isolate from snap bean). Species of *A. euteiches* are genotypically and phenotypically heterogeneous (Malvick and Percich, 1998a). Races exist within these species and are a result of sexual recombination. These races interact differentially with host varieties. Improved understanding of the variation of virulence within *A. euteiches* would aid in the development of control strategies that employ crop rotation and host resistance.

2.2.7 Physiologic Specialization/ Host Specificity

The existence of physiologic specialization in *A. euteiches* was demonstrated when King and Bisonette (1954) found differences in the pathogen's ability to attack different varieties and selections of peas. Scharen (1960) obtained similar results. Beute and Lockwood (1967) published the most comprehensive study on physiologic specialization within *A. euteiches*. They inoculated 6 pea varieties with 15 isolates of the fungus. Based on virulence patterns found on the six differential pea varieties, two races of *A. euteiches* were designated: races 1 and 2. Using the same six pea varieties, Sundheim (1972) furthered the study by outlining three additional physiologic races: races 3, 4 and 5. The existence of distinct physiologic races of *A. euteiches* must be considered in programs aimed at breeding peas for resistance to the fungus (Sundheim, 1972).

2.2.8 Distribution

Aphanomyces euteiches is a destructive pathogen in pea-growing areas all over the world (Kraft and Boge, 1994; and Pfender, 1984). Wicker *et al.* (2001) stated that *A. euteiches* had been identified in many regions of the Midwest, Central and North-Western states of the USA (Holub *et al.*, 1991b; Kraft and Boge, 1994), Canada (Basu *et al.*, 1973), Europe (Persson *et al.*, 1997), Australia (Allen *et al.*, 1987), New Zealand (Manning and Menzies, 1980) and Asia (Yokosawa *et al.*, 1974), causing heavy losses, primarily on pea crops. Conners (1967) reported that *A. euteiches* caused root rot of pea in Eastern Canada (Quebec, Ontario) but considered the pathogen to be unimportant in Ontario. An extensive study on the incidence of *A. euteiches* in Manitoba has yet to be

undertaken. One documented disease survey of Manitoba, conducted by Mathur *et al.* (1998) in 1997, concluded that over 30% of diseased pea fields surveyed in southeast and central regions were a result of Aphanomyces root rot.

2.3 Environmental Effects

The role of the environment in the development of plant disease is obvious: environment influences both the host and the pathogen, host and pathogen affect each other, the host often changes the environment, but the pathogen rarely does (Zadoks and Schein, 1979). Changes in the environment can be brought on as a result of other organisms, tillage practices, or climatic conditions and may result in an altered growth response of the pathogen. The environment in which soil organisms exist is complex, involving numerous independent but interacting factors. Some of these factors include temperature, moisture and soil.

2.3.1 Temperature

Temperature affects both reproduction of and infection by the pathogen. Howard *et al.* (1994) found that *A. euteiches* required low soil temperatures (14-20°C). However, high temperatures were also found to favour infection. The minimum temperature for infection by *A. euteiches* was determined to be 10°C (Smith and Walker, 1941).

2.3.2 Moisture

Moisture is the primary limiting factor of this disease (Howard *et al.*, 1994). The pathogen relies on moist conditions to disperse via swimming zoospores in order to infect a host. If conditions are very dry, the chance of infection decreases greatly. Burke *et al.* (1969) reported that brief periods of soil saturation, which might occur with several hours of rain, were sufficient enough to promote infection in seedlings. Plants became infected when moisture levels were close to field capacity.

2.3.3 Soil

Soil properties also contribute to the prevalence and intensity of *A. euteiches* infection. Disease is generally more severe in wet seasons, at soil temperatures of 24-28°C, and in soils with a high water capacity (Temp and Hagedorn, 1967). No correlation was found between soil type or water-retaining capacity of the soil and Aphanomyces root rot, except with clays (Walker and Hare, 1943). Temp and Hagedorn (1967) found a positive relationship between soil types with a high water-retaining capacity (red clay, gray-yellow silt loam, muck) and slower decreases in the root rot potential.

2.4 Disease Control

Crop rotation, biological control agents, fungicides and host resistance have been investigated to manage root rot caused by *A. euteiches* (Pfender, 1984; Papavizas and Ayers, 1974). Disease is primarily reduced through crop rotation and/or avoiding fields with high infestation levels (Malvick and Percich, 1999). Bowers and Parke (1993)

stated that there were no commercially available pea varieties with resistance to Aphanomyces root rot or fungicides effective in suppressing the disease. All fields have at least some level of root rot potential. Practices that might result in increasing a pea crops susceptibility to this disease must be avoided (Harvey *et al.*, 1975). Currently, the only real mode of "control" is the avoidance of fields infested with the fungus.

2.4.1 Avoidance

Avoiding infested fields is the basis of root rot control (Jacobsen and Hopen, 1981). Harvey *et al.* (1975) reported that the only effective method of reducing crop loss is to avoid fields that posed the most serious disease threat since attempts to control this disease by using chemicals, crop rotations or by breeding resistant varieties had not been successful.

2.4.2 Crop Rotation

Pfender (1984) stressed the need for long rotations with non-host crops between pea crops for safe pea production. Holub *et al.* (1991a) stated that a relationship had been found between the cropping history of agricultural soils and the host specialization of *A. euteiches* isolates collected from those soils. Host-range is agronomically important because crop rotation is generally used as a means of controlling the induced diseases (Papavizas and Ayers, 1974). Reducing the number of host crops seeded to a contaminated field would help to reduce the build-up of inoculum. However, oospores can exist in the soil for long periods with the ability of some germinating while others remain dormant. This is believed to be the reason for *A. euteiches* survival success.

If certain crops grown in rotation with pea help to accelerate a decline in the root rot potential of a field, then they could be emphasized in the rotation to achieve at least some degree of control (Temp and Hagedorn, 1967). Temp and Hagedorn (1967) also reported that *A. euteiches* persisted in soils even after 6-8 years without peas, emphasizing the importance of not planting pea in fields with a high root rot potential. Pfender and Hagedorn (1983) determined that, in sandy soils, inoculum levels decreased 50% in 1 year in the absence of pea. Therefore, if the half-life of inoculum is considered to be 1 year, then 9 years without peas would be needed to decrease inoculum levels from 3.6 infective propagules/gram (severe shoot symptoms and yield loss of about 85%) to 0.006 infective propagules/gram (mild shoot symptoms and about 40% yield loss). This agrees with earlier findings by Temp and Hagedorn (1967) that 6-8 years without pea was required.

2.4.3 Biological Control

Biological control involves the direct use of negative interactions: pathogenesis, competition, antibiosis, or antagonism to regulate the population of a pathogen or pest (Zadoks and Schein, 1979). This competition among organisms may be directed toward the control of plant pests by the introduction of a natural parasite or predator of the pest (Janick, 1986). In plant pathology, there is evidence that one organism can negatively affect the population level of another which is pathogenic (Zadoks and Schein, 1979). These organisms include: bacteriophages and vibroid bacteria against bacteria, bacteria against fungi, viruses against fungi, viruses against viruses, fungi against fungi and fungi against parasitic phanerogams and weeds (Zadoks and Schein, 1979).

Experimental biocontrol of *Pythium* and *A. euteiches* f. sp. *pisi* has been obtained by treating the seed with liquid suspensions of living biocontrol agents before planting (Parke *et al.*, 1991). However, this method was not found to be practical for large-scale applications because of the need to formulate bacterial and fungal spore suspensions immediately before application.

The genus *Gliocladium* has been regarded as the slimy counterpart of *Penicillium* but with the same mechanism of conidium formation (Thom, 1910, 1930). *Gliocladium roseum* is a common soil fungus and decomposer of rotting plant material worldwide (Domsch *et al.*, 1980). With high competitive saprotrophic capacities, *G. roseum* is a destructive (necrotrophic) mycoparasite (Barnett and Lilly, 1962) that can kill numerous fungal species by hyphal penetration (Turhan, 1993).

Xue (2002) reported that the bioagent ACM941, a strain of *Gliocladium roseum* Bainier, was effective in controlling pea root rot caused by *A. euteiches*, *F. oxysporum* f.sp. *pisi*, *F. solani* f.sp. *pisi*, *M. pinodes, Pythium* spp., and *R. solani.* Xue (2002) also states that the use of ACM941 to control these fungal pathogens was an effective alternative to existing chemical products, illustrating increased levels of efficacy, prolonged periods of protection, a broader range of pathogenic targets and improved environmental safety.

2.4.4 Chemical Control

Fungicides

There are two types of fungicides; protective and eradicant (Zadoks and Schein, 1979). Protective fungicides control infections that will take place in the future. These

remain on the outside of the plant and are referred to as contact fungicides. Eradicant fungicides control infections that have already taken place. These are systemic fungicides that are taken up by the plant and transported throughout.

Currently, there are no chemicals registered for control of *A. euteiches* in pea. Mitchell and Hagedorn (1971) reported the use of fenaminosulf (Lesan, formerly Dexon) for control of *A. euteiches*. This fungicide was effective and labeled for use but was not considered economical (Jacobsen and Hopen, 1981).

Schwinn (1983) reviewed the efficacy of several new types of fungicides showing promise for marked improvement in chemical control. The author found that prothiocarb/propamocarb, under the trade name Previcur S70 or Previcur N, exhibited variable activity against *Aphanomyces* while metalaxyl, under the trade names Ridomil, Acylon and Apron, exhibited no useful activity against the pathogen.

Although fungicide seed treatments have been relied upon for years for the suppression of root rot pathogens in pea, they are not desirable for disease control due to some adverse effects on the environment and ecosystem, such as harm to non-target organisms, animals, and plants, soil residues and contamination of the water and food chains (Xue, 2002). Cook and Baker (1983) reported that fungicides may induce pathogen resistance, making their effects variable and short lived. Xue (2002) also addressed the issue of cost, in that fungicides are expensive in comparison to the relatively low commodity price of field pea.

Herbicides

There are some herbicides that reduce the incidence of plant disease. These herbicides include: dimethylolpropionic acid (DMPA) which inhibits *Pythium debaryanum* in peas (Fields and Hemphill, 1967), dinodeb which inhibits *Cercospora arachidicola* in peanuts and 2,4-D, Oropham and dinoseb which inhibited *Fusarium oxysporum* and *F. lycopersici* in tomatoes. At higher concentrations, most herbicides are fungitoxic (Harvey *et al.*, 1975).

Jacobsen and Hopen (1975) reported that dinoseb effectively lowered pea root disease caused by *A. euteiches*. Higher yields were also reported when peas were grown in soils treated with trifluralin (Harvey et al., 1975). Carlson and Hopen (1971) reported that pea root rot was less severe where trifluralin (Treflan, Elanco) was applied to the soil for weed control. Trifluralin is believed to suppress the motility of zoospores. Commercial pea growers can use trifluralin or dinitramine without risk of increasing root disease incidence when *A. euteiches* is the primary cause of root rot (Grau and Reiling, 1977).

In some instances, herbicides have influenced the incidence of plant disease. These herbicides include: atrizine which increased root and epicotyl rot caused by *F. solani* f. sp. *pisi* (Percich and Lockwood, 1975), trifluralin and nitralin which increased *Rhizoctonia solani* in cotton, prometryne that increased *Sclerotium bataticola* in cotton, and 2,4-D and dalapon which increased disease in tomato. Atrazine, Eptan 8-E and Trifluralin where shown to stimulate various pathogenic fungi at certain concentrations.

2.4.5 Disease Resistance

Grittton *et al.* (1995) reported that the ability of a plant to survive and produce grain yield is the ultimate test of its resistance or tolerance to root rot or the efficacy of a control treatment. Different levels of resistance to Aphanomyces root rot exist between pea cultivars (Malvick and Percich, 1998a). Therefore, the greatest benefit at present would seem to come with the choice of pea genotype. Gritton *et al.* (1995) stated that the best genotype for a given location varies due to differences in the *Aphanomyces* biotype present and the environment.

Breeding resistance to Aphanomyces root rot in pea has been difficult to achieve due to challenges in identifying and incorporating useful disease resistance to populations of *A. euteiches* (Beute and Lockwood, 1967; Grau *et al*, 1991; Malvick *et al.*, 1998; and Malvick and Percich, 1998b). Marx *et al.* (1972) reported that resistance to common root rot is associated with three dominant alleles that control node length, flower colour and hilum colour. Substitution of the recessive horticulturally desirable alleles resulted in reduced resistance. Resistance level is generally low and the incorporation of this resistance into commercial cultivars would necessitate a lengthy backcrossing program (Yeoman, 1986). Variability within populations of *A. euteiches* may also cause inconsistent performance of disease-resistant pea lines in the field (Grau *et al.*, 1991; Malvick *et al.*, 1998; Malvick and Percich, 1998b; and Sundheim, 1972).

Chapter 3.0

Geographic Distribution, Pathogenicity and Host-range of *Aphanomyces euteiches* in Manitoba

3.1 Abstract

Determining the incidence, pathogenicity and host-range of A*phanomyces euteiches* root rot would not only assist in disease management, but provide knowledge of the pathogen to breeding programs aiming to produce resistant varieties. To determine the incidence of *A. euteiches* in Manitoba, a survey of commercial fields was undertaken in 1999 and 2000. Forty-four pea fields in Central and Southern Manitoba were surveyed. Growth cabinet experiments were conducted in 2000 and 2001 to determine the environmental conditions and hosts favourable to the pathogen. Using the pea variety Carneval, seven *A. euteiches* isolates were studied at four growing temperatures (16, 20, 24, and 28°C) and three seedling ages (1, 2, and 3 weeks). To determine the host-range of the pathogen from Manitoba, seven isolates from different fields were tested against four pea varieties (AC Tamor, Carneval, Marjoret, and Trapper), eight lentil varieties (CDC Glamis, CDC Robin, Crimson, Eston, French, Indianhead, Laird, and Richlea), two chickpea varieties (CDC Desiray and CDC Yuma), six bean varieties (AC Clack Diamond, CDC Pintium, AC Scarlet, Envoy, Navigator, and Pintoba), one alfalfa variety (OAC Minto) and one soybean variety (Alta). *Aphanomyces euteiches* was isolated from nine of the 44 fields surveyed. These fields ranged from Morden to Russell, Manitoba. Disease severity and root and shoot dry weights were significantly affected by growing temperatures of 16, 20, 24 and 28°C. These results suggest that infection and disease development of *A. euteiches* were favourable at all four temperature regimes used in this experiment.

Seedling age at time of inoculation did not consistently affect disease development. Pea, lentil and alfalfa were the only crops infected by the *A. euteiches* isolates. Lentil and alfalfa were the only crops to exhibit differential infection patterns. Chickpea, bean and soybean all exhibited immunity to the isolates. Survey results suggest that the incidence of *A. euteiches* in Manitoba is greater than previously reported. Pathogenicity tests also suggest that virulence and host-range varied among Manitoba's Aphanomyces isolates.

3.2 Introduction

With increased production of field pea (*Pisum sativum* L.) in Manitoba, there is intense interest in the distribution, pathogenicity and host-range of *Aphanomyces euteiches* Drechs. Currently, there are no commercially available root rot resistant pea varieties or biological or chemical controls effective in suppressing the disease (Gritton *et al.*, 1995). Disease is reduced primarily through crop rotation and avoidance of fields highly infested with *A. euteiches* (Malvick and Percich, 1999). Determining one or all of these factors will help uncover some of the mystery behind *A. euteiches* success as a root rot pathogen in field pea, but will also play an important part in the integrated approach to existing control measures.

Root rot caused by *A. euteiches* has been reported in most pea-growing areas of North America, Northern Europe, Australia, New Zealand and Japan (Pfender, 1984). Distribution of this pathogen in Manitoba is relatively unknown. To date, *A. euteiches* has only been reported in the Red River Valley on faba bean (Lamari and Bernier, 1985) and pea (Mathur *et al.*, 1998). Determining *Aphanomyces* distribution in Manitoba will help increase awareness of the disease and enable producers to implement crop rotations that do not favour disease development.

The host-range of *A. euteiches* is of great interest because crop rotation is one of the few means of controlling this soil-borne plant pathogen (Papavizas and Ayers, 1974). *Aphanomyces euteiches* infects a variety of legume species (Papavizas and Ayers, 1974). Linford (1927) was the first to report a possible pathogenic relationship between *A. euteiches* and a crop other than pea. Pea is the crop that appears to be the most seriously damaged by Aphanomyces root rot, but alfalfa (*Medicago sativa* L.), snap bean

(*Phaseolus vulgaris* L.), red clover (*Trifolium pratense* L.) (Malvick and Percich, 1998a), and faba bean (*Vicia faba* L.) (Lamari and Bernier, 1985) are also crops which may be severely damaged. A better understanding of the pathogenic variation of *A. euteiches* would aid in the development of control strategies that employ crop rotation and host resistance, ultimately reducing field inoculum levels and disease severity (Grau *et al.*, 1991).

The objective of this study was to determine the incidence of *A. euteiches* in Manitoba, as well as virulence and host-range differences which may exist among the isolates.

3.3 Materials and Methods

3.3.1 Incidence of *A. euteiches* in Manitoba

A survey of pea fields in Manitoba was conducted during the summers of 1999 and 2000. Forty-four fields within Manitoba were randomly selected and surveyed for the presence of *A. euteiches* (Appendix 2.1). Approximately twenty shovels of soil were collected and bulked from each field. Samples were randomly taken from low lying moist areas. Soil was collected for the purpose of pathogen baiting and isolation.

3.3.2 Seedling Production

Nine-centimeter plastic pots were soaked in a strong bleach solution, scrubbed and rinsed thoroughly to reduce the occurrence of contamination from sources other than the isolate in question. One filter paper was placed in the bottom of each pot to ensure no vermiculite (Terra-Lite®2000) ran through the drainage holes. Pots were filled ¾ full

with vermiculite. Seeds were surface sterilized for 15 seconds using a 0.5% NaOCl solution, rinsed in sterile distilled water and blotted dry using a sterile paper towel. Ten pea seeds of the cultivar Carneval were sown in each pot. Seeds were covered with approximately 2.5 cm of vermiculite and fertilized using 20-20-20 (1Tbls/Gal). Once plants emerged, they were thinned to eight plants per pot. Plants were watered and fertilized as required. Plants were grown in a Watlow 1500-Environment Growth Chamber with a 16 hour photoperiod and a temperature of 20° C.

3.3.3 Isolation, Sporulation and Inoculation of *A. euteiches*

Isolation

Soil samples collected from survey sites were used to fill 9-cm plastic pots and seeded with the pea variety Carneval. When seedlings were 10-14 days old, they were gently uprooted. Roots exhibiting typical *A. euteiches* symptoms were thoroughly washed, the cortical tissue mounted on microscope slides and examined immediately under a compound microscope (Zeiss, Oberkuchen, Germany), for the presence of oospores (Figure 3.1 a-b). Alternatively, some roots were rinsed under running cold water for 24 hours. Bacterial contamination was reduced and *A. euteiches* recovery was improved with this rinsing procedure (Lamari and Bernier, 1985). They were then surface-sterilized using a 0.5% NaOCl solution, rinsed with sterile distilled water, cut into 3 to 5 mm sections and plated onto water agar (WA, Difco, Kansas City, Missouri) (Appendix 1.1). Plated sections were incubated at 24°C for two days and observed frequently for the presence of bacterial/fungal contamination. Hyphal tips were sub-

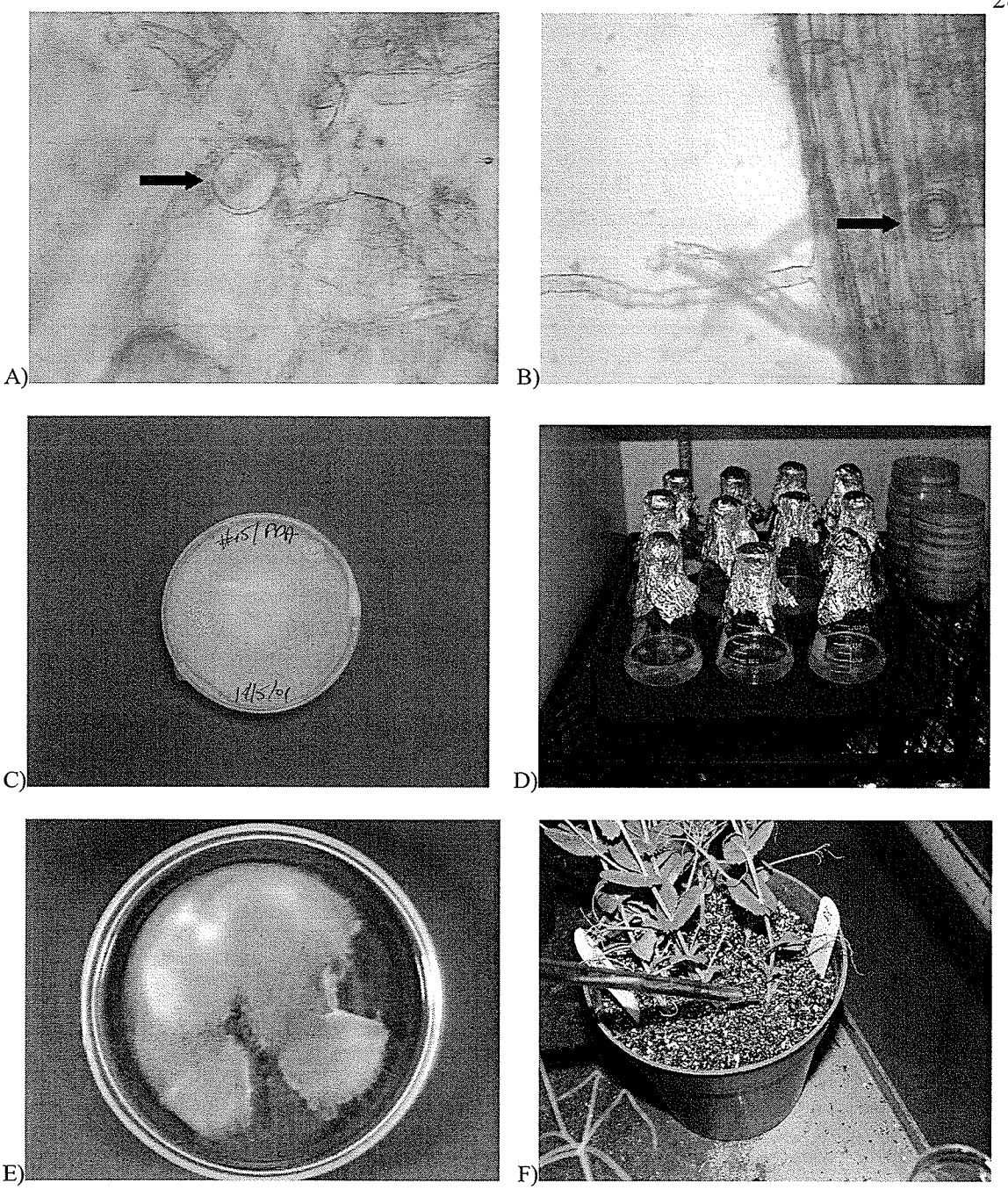

Figure 3.1. *Aphanomyces euteiches* isolation, sporulation and inoculation. A-B) Oospores embedded in the cortical tissue of field pea, 100x. C) *A. euteiches* culture growing on potato dextrose agar. D) Cultures of *A. euteiches* soaking in sporulation rinse solution. E) Five day old mycelial mats. F) Inoculation procedure of *A. euteiches*. Two ml of $1x10^4$ spores/ml spore suspension are pipetted at the base of the pea seedling.

cultured onto potato dextrose agar (PDA, Difco, Appendix 1.2) plates and incubated for another four to eight days (Figure 3.1 c). Pure cultures were examined under a compound microscope for the presence of oospores characteristic of *A. euteiches*. One isolate/field was selected and labeled according to the field number (15, 22, 24, 25, 26, 27, and 41) from which they were recovered. Isolates were stored at 4°C until used in other experiments.

Sporulation

Zoospores of *A. euteiches* were produced using the methods of Llanos and Lockwood (1960), with slight modifications. Only pure *A. euteiches* cultures obtained from hyphal tips were used. Mycelial plugs of the fungus were grown in 250 ml Erlenmeyer flasks containing 50 ml of Maltose (3%) Peptone (1%) broth (Appendix 1.3). The cultures were incubated for five days at 24°C without agitation. Mycelial mats were then rinsed using a sporulation wash solution (Mitchell and Yang, 1966) (Figure 3.1 d-e, Appendix 1.4). Two additional rinses were performed at one hour intervals. Following the last rinse, sporulation occurred at different time intervals for different cultures (Figure 3.2 a-c). The average time required for sporulation was determined to be approximately 16 hours (Appendix 3.1). Spores were immobilized by adding a drop of Javex (sodium hypochlorite 6%) to the spore sample. Once immobilized, spore concentrations were determined using a hemacytometer and adjusted to $1x10^4$ spore ml^{-1} using the sporulation wash solution of Mitchell and Yang (1966).

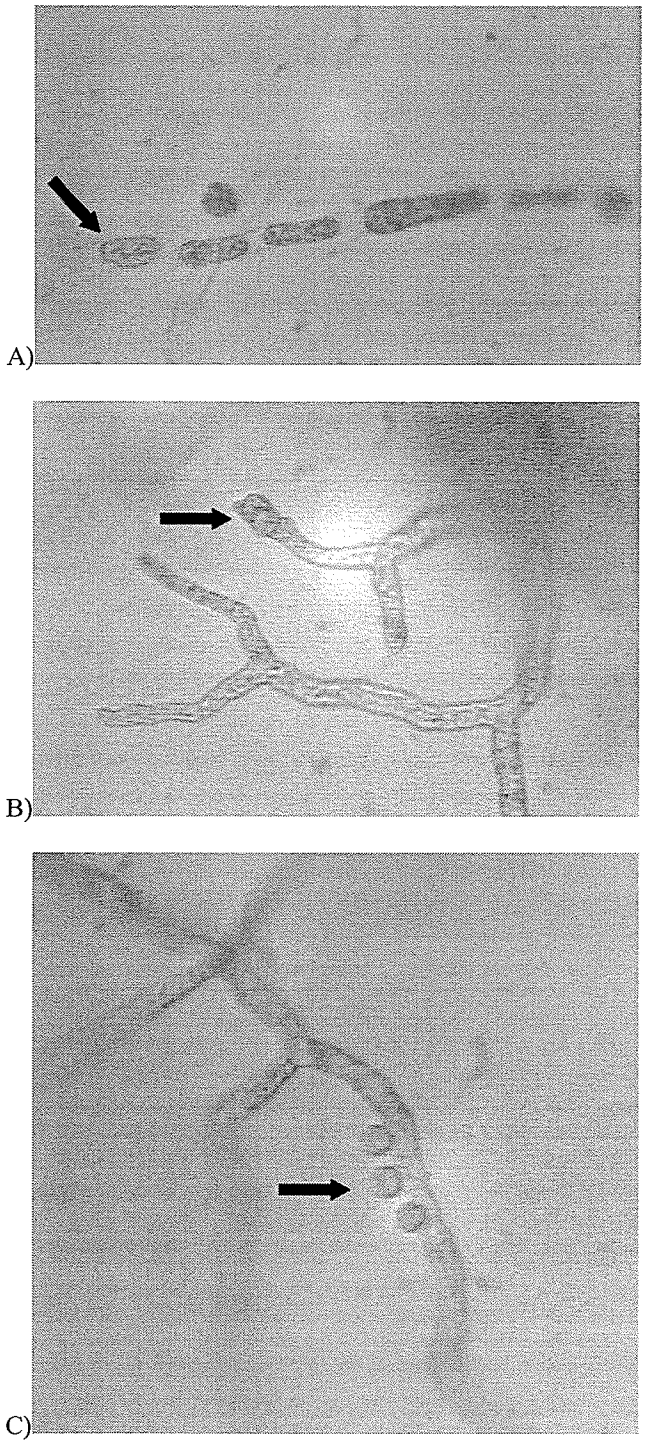

Figure 3.2. Microscopic observations of *Aphanomyces euteiches*. A) Differentiation of cytoplasm within the hyphae, 100x. B) Zoospore release and encystment at the tip of an undifferentiated sporangiophore, 100x. C) Released zoospores next to aseptate hyphae, 100x.

Inoculation

The vermiculite at the base of each seedling was gently removed in order to expose the epicotyl and root system. Using a pipette, 2 ml of the spore suspension was applied to the epicotyl and/or root system of each seedling (Lamari, 1982) (Figure 3.1 f). The vermiculite was replaced once inoculation was completed. Inoculated plants were incubated in a growth cabinet for 14 days at temperatures specified by the individual experiments. Plants were watered and fertilized as required.

Inoculum for the host-range experiment was prepared by growing each *A. euteiches* isolate on a plate of PDA (Figure 3.1 c). Using a scalpel, the plated cultures were divided into 3x3 mm sections. Seedlings were inoculated by inserting one of these mycelial sections against the epicotyl and/or root system of each plant. Once inoculated, the plants were incubated in a growth cabinet for 21 days at 24° C. Moisture and fertility were maintained during this period.

Two different inoculation procedures were used in the pathogenicity and host-range studies. Pathogenicity tests were conducted using known concentrations of zoospores for the purpose of quantifying the disease and to ensure that the pathogen could infect pea plants in a manner similar to that observed under field conditions. Mycelial plugs were used in the host-range study to ensure that the pathogen was in direct contact with the host to avoid the possibility of escape from infection. We were seeking in this study to determine if the plant species tested were host or non-host to the pathogen (ie: a qualitative response).

3.3.4 Pathogenicity

At specified seedling ages (7, 14 and 21 days), plants were inoculated using zoospore suspensions of *A. euteiches* isolates 15, 22, 24, 25, 26, 27 and 41. Four growth room temperatures were tested in this experiment (16, 20, 24 and 28° C). Once inoculated, the plants were returned to the growth cabinet where moisture and fertility were maintained. Disease severity was recorded 14 days post-inoculation. Treatments were replicated three times and the whole experiment was repeated once.

3.3.5 Host-range

A total of 14 host varieties, including pea (AC Tamor and Majoret), lentil (CDC Glamis and CDC Robin), chickpea (CDC Desiray and CDC Yuma), bean (AC BlackDiamond, AC Scarlet, CDC Pintium, Envoy, Navigator and Pintoba), alfalfa (OAC Minto) and soybean (Alta) were tested in this experiment (Table 3.8). Eight pots of each variety were seeded. This allowed for a non-treated control and seven isolates to be tested. The seven isolates included 15, 22, 24, 25, 26, 27 and 41. Pots were over-seeded and thinned to seven plants per pot once the seedlings had emerged. The experiment had four replicates (1 pot=1 replicate). Due to unclear infection results on the alfalfa variety and selective pathogenicity on the lentil varieties, both within and among isolates, the experiment was repeated once. However, the second experiment included the same alfalfa and lentil varieties used in the first, plus six additional lentil varieties (Crimson, Eston, French, Indianhead, Laird and Richlea). The additional lentil varieties were incorporated in the experiment in an attempt to determine further pathogenicity differences which may exist within and among the seven *A. euteiches* isolates.

Fourteen-day-old seedlings were inoculated, as described above, and returned to a 24° C growth cabinet. Pathogenicity was recorded 21 days post-inoculation.

3.3.6 Disease Assessment

Plant reaction was scored according to Sherwood's (1958) 0-4 system of rating (Figure 3.3 a-f). The disease classes employed were as follows: 0 - Healthy roots; 1 - Roots with a few water-soaked, light brown areas; 2 - Roots water-soaked, light brown areas confluent and more extensive but not involving the entire root system; 3 - Water-soaked and browning involving all roots and epicotyl (stem above seed piece), tissue soft but not collapsed, epicotyl not markedly shriveled; 4 – Water-soaked, browning and decay involving all roots and epicotyl, cortex easily sloughed off, epicotyl shriveled or rotted.

Disease severity was determined by up-rooting all plants from the vermiculite, rinsing the root system free of debris and visually rating the plants based on the symptoms present on the epicotyl and root system. Isolations were made from the infected root tissue to confirm the presence of *A. euteiches*.

Measurements taken to determine virulence characteristics for each of the seven *A. euteiches* isolates included: disease severity and dry root and shoot weights. Root and shoot sections were dried separately for 48 hours at 80°C before being weighed. Disease severity and dry weight data were analyzed using SAS (SAS Institute Inc., Cary, NC, USA).

To determine the host-range of all seven *A. euteiches* isolates, plant roots were assessed visually for the presence or absence of infection. Root tissue was also examined

under a compound microscope for the presence of oospores. A host was only considered susceptible if there was visual evidence of infection on the roots and oospores present in the root tissue.

3.4 Results and Discussion

3.4.1 Incidence

Forty-four fields throughout Manitoba were surveyed for the presence of *A. euteiches* (Appendix 2.1). *Aphanomyces euteiches* was isolated from nine of forty-four fields (Table 3.1 and Figure 3.4). Other pathogens isolated from these fields include *Fusarium* spp. (48% of fields), *Pythium* spp. (23% of fields) and *Rhizoctonia solani* (9% of fields). This is consistent with earlier reports of fungi associated with root rot of pea(Papavizas and Ayers, 1974; Tu, 1987; Xue, *2002).* This is also consistent with Reiling *et al.* (1960) who reported *A. euteiches* and *F. solani* to be the most prevalent root rotting organisms in infested fields.

The origin of the *Aphanomyces* isolates ranged from as far south as Morden to as far north as Russell (Figure 3.4), a distance of approximately 400 km (Manitoba Transportation and Government Services, 2004). Previous reports on Aphanomyces root rot of pea were from within the Red River Valley (Mathur *et al.,* 1998, Xue, 2002). Sites harbouring *A. euteiches* were plotted on a map to help illustrate its distribution in Manitoba, as determined by this survey (Figure 3.4). These results suggest that *A. euteiches* is more extensively spread in Manitoba than previously reported.

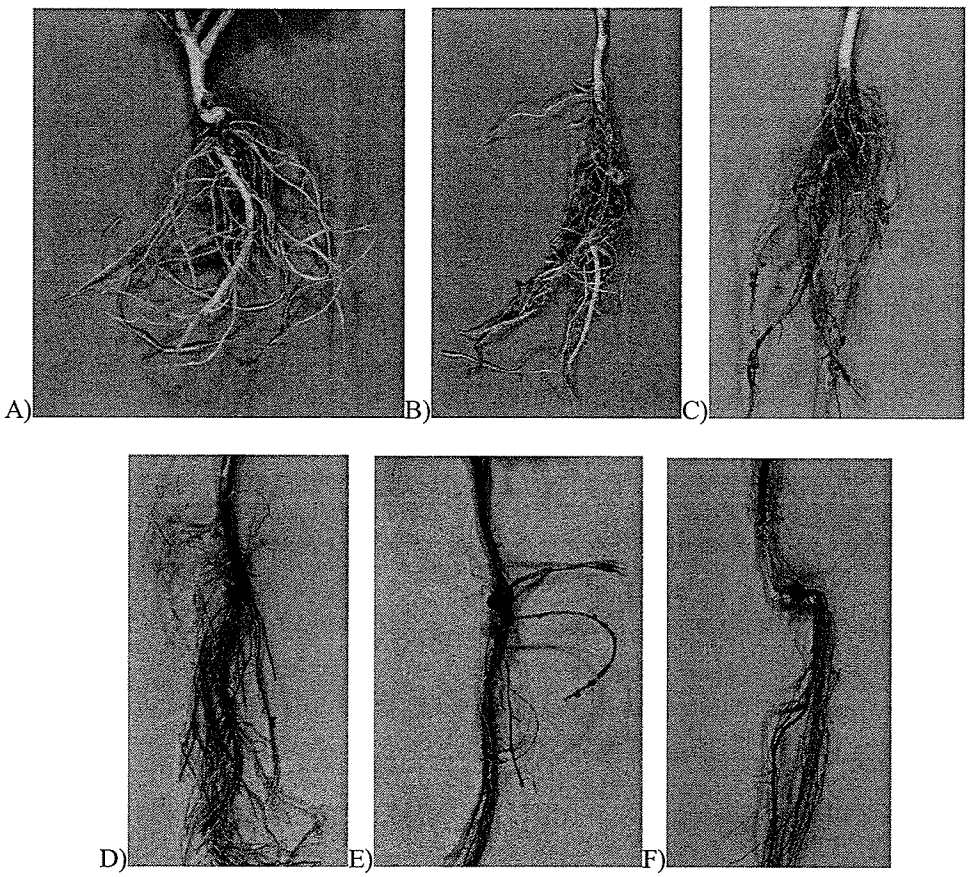

Figure 3.3. Sherwood's 0-4 system of rating (1958) demonstrated on field pea roots. A) 0 - Healthy roots. B) 1 - Roots with a few water-soaked, light brown areas. C) 2 - Roots water-soaked, light brown areas confluent and more extensive but not involving the entire root system. D) 3 - Water-soaked and browning involving all roots and epicotyl (stem above seed piece), tissue soft but not collapsed, epicotyl not markedly shriveled. E and F) 4 – Water-soaked, browning and decay involving all roots and epicotyl, cortex easily sloughed off, epicotyl shriveled or rotted.

Table 3.1. A list of the nine survey site locations in Manitoba where *Aphanomyces euteiches* isolations originated.

Field/ID#	Location	Pathogens Isolated			
		A. euteiches	*Rhizoctonia*	*Fusarium*	*Pythium*
5	259, West of 25, South side	.+		.+	
15	Hwy 83 at Russell, MB, West side	.+	.+	.+	
22	432, 2 miles South of 23, West side	.+		.+	
23	432, 1 mile South of 23, West side	.+		.+	
24	Hwy 3, 1 mile East of Carman, MB, North side	.+		.+	.+
25	*Morden Research Station	.+		.+	
26	*Penner's, Hwy 3 North of Morden	.+		.+	
27	*Winnipeg, U of M, Point block 18	.+		.+	
41	248, 2.5 miles North of Hwy 1	.+		.+	

* 1999/2000 Treatment trial sites.

3.4.2 Pathogenicity

Analysis of variance tables are provided in Appendix 4.

Isolate

Significant differences (p=0.05) in pathogenicity, as measured by disease severity, were observed between the seven *A. euteiches* isolates tested (Table 3.2). Similar results were obtained by King and Bisonette (1954), who were the first to demonstrate pathogenic variability in six isolates of *A. euteiches*. In both experiments, disease severity was the highest for isolate 41 (3.08 and 2.37), whereas the non-treated control exhibited no disease. However, ranking of significance for the remaining isolates were inconsistent between experiments; suggesting that those isolates caused similar levels of disease.

Root and shoot dry weights were significantly different in experiment 1, but only slightly significant in experiment 2 (Tables 3.3-4). In both experiments, the non-treated control (2.86 and 2.12 g) had the highest RDW and isolate 41 had the lowest (1.99 and 1.65 g). In experiment 1, the non-treated control (3.26 g) had the highest SDW and isolate 41 had the lowest (2.76 g). Again, the overall ranking of significance for the remaining isolates was inconsistent between experiments.

Temperature

Disease severity was significant (p=0.05) with temperature change (Table 3.5). However, significance rankings were different between experiments. These results suggest that disease development was favourable at all four temperature regimes tested. This supports

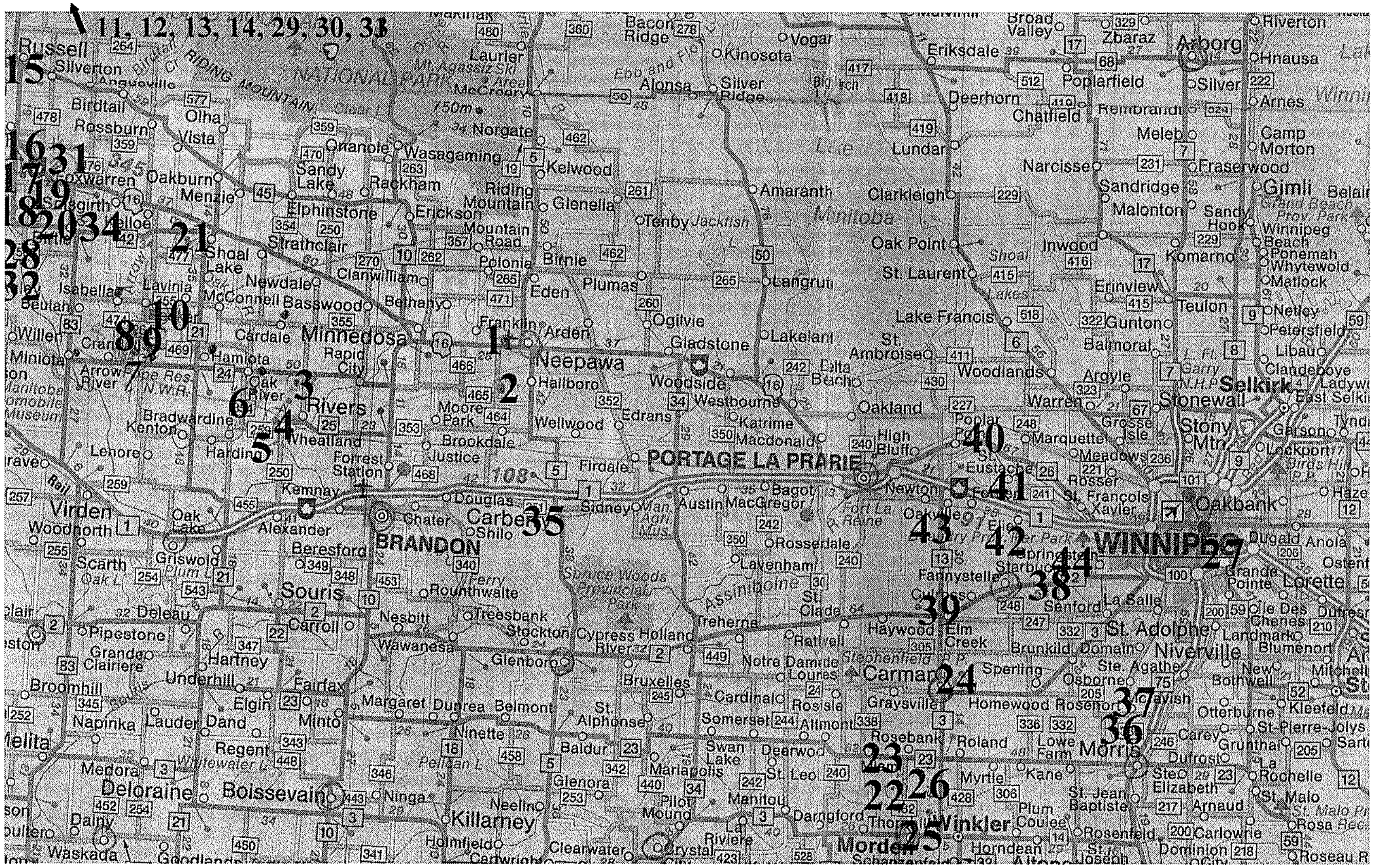

Figure 3.4. A partial map of Manitoba illustrating the 44 disease survey sites. Sites with blue labels represent sites where *Aphanomyces euteiches* was isolated.

findings by Smith and Walker (1941), who reported temperatures of 16-28°C to be favourable for infection and subsequent disease development by *A. euteiches*.

Root and shoot dry weights were significantly different (p=0.05) with temperature change (Tables 3.6-7). Significance rankings were consistent between experiments, for both. Root and shoot dry weights were the lowest at 28°C, whereas, root and shoot dry weights were the highest at 24 and 16°C, respectively. Burke *et al.* (1969) reported that the incidence of initial infection of the taproot was slightly greater at 28°C than at 16°C. These findings may explain the lower root and shoot dry weights reported at 28°C in this study.

Seedling Age at Time of Inoculation

Seedling age had no affect on disease severity or root dry weight. Shoot dry weight differed significantly (p=0.05) with seedling age (Appendix 5.1-3). However, significance rankings were inconsistent between experiments, suggesting that the seedling ages used in this study had similar effects on shoot dry weight.

3.4.3 Host-range

Testing only for pathogenicity, the virulent and avirulent reactions produced by the seven *A. euteiches* isolates on each host are outlined in Table 3.8. Susceptible reactions were only confirmed if visual symptoms were accompanied by microscopic observation of oospores in the root tissue. Carneval and Trapper were included in this test because they were the pea varieties used throughout this study to bait the pathogen and have known susceptibility reactions to all seven isolates.

All seven *A. euteiches* isolates produced susceptible reactions on the four pea varieties tested and resistant reactions on the soybean, chickpea and bean varieties tested (Table 3.8). These results suggest that the isolates were restricted to the f. sp. *pisi* as a result of their virulence to pea and avirulence to bean. Strains of *A. euteiches* with selective pathogenicity to bean and pea were proposed to be sufficiently host-specific to be recognized as *forma specialis phaseoli* and *pisi*, respectively (Pfender and Hagedorn, 1982). Therefore, one might assume that the isolates obtained were f. sp. *pisi*. However, reports by Grau *et al.* (1991) and Holub *et al.* (1991b) suggest that f. sp. *pisi* is not sufficiently distinct from other biotypes to justify a *forma specialis* designation.

Lentil and alfalfa were the only hosts to have both susceptible and resistant lines to the seven isolates. Several researchers have associated *A. euteiches* with seedling blight of alfalfa (Linford, 1927; Sherwood and Hagedorn, 1962; Schmitthenner, 1964; McKeen and Traquair, 1980; and Delwiche *et al.*, 1987). Similarly, results by Linford (1927) and Sherwood and Hagedorn (1962) were based on studies of host-range using isolates collected from pea. Forty percent of the isolates (22, 24, and 27) exhibited pathogenicity on both pea and alfalfa (Table 3.8). These results are similar to reports by Holub *et al.* (1991), who found that 80-100% of isolates from pea root rot soils and pea/alfalfa root rot soils were moderately to highly virulent to both hosts. Holub et al. (1991b) also reported that the wide spectrum of virulence to pea and alfalfa illustrated an ambiguity which exists in the intraspecific taxonomy of *Aphanomyces euteiches*. The virulence observed on lentils in this study may further illustrate the ambiguity which exists in the host-range of the pathogen. Six of the seven isolates used in this study

Table 3.2. Effect of *Aphanomyces euteiches* isolate on disease severity (DS).

Experiment 1			Experiment 2		
Isolate	DS[1]		Isolate	DS	
41	3.1	a[2]	41	2.3	a
25	2.5	b	27	2.0	ab
15	2.3	bc	25	2.0	ab
27	2.3	bc	26	1.8	bc
24	2.2	bc	22	1.8	bc
22	2.2	bc	24	1.6	c
26	2.1	c	15	1.6	c
Control	0.0	d	Control	0.0	d
LSD	0.373		LSD	0.438	

[1] Average disease severity of 8 plants/pot, 3 replications.

[2] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 3.3. Effect of *Aphanomyces euteiches* isolate on root dry weight (RDW).

Experiment 1			Experiment 2		
Isolate	RDW[1] (% control)		Isolate	RDW (% control)	
Control	100.0	a[2]	Control	100.0	a
26	85.3	b	25	97.6	a
24	79.7	bc	15	94.3	a
22	76.9	bc	22	92.9	a
27	76.6	bc	26	90.6	ab
15	76.2	bc	24	90.1	ab
25	70.3	c	27	88.2	ab
41	69.6	c	41	77.8	b
LSD	14.7		LSD	13.5	

[1] Average root dry weight (8 plants/pot, 3 replications) as a percent of the control. Determined by multiplying the average RDW by the average RDW of the non-inoculated control and multiplied by 100.

[2] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 3.4. Effect of *Aphanomyces euteiches* isolate on shoot dry weight (SDW).

Experiment 1			Experiment 2		
Isolate	SDW[1] (% control)		Isolate	SDW (% control)	
Control	100.0	a[2]	15	103.9	a
22	96.3	ab	22	103.6	ab
27	94.5	ab	25	101.0	ab
26	93.3	ab	41	100.0	ab
24	91.7	b	Control	100.0	ab
15	90.5	bc	26	99.7	ab
25	89.9	bc	27	99.0	ab
41	84.7	c	24	96.7	b
LSD	6.75		LSD	7.26	

[1] Average shoot dry weight (8 plants/pot, 3 replications) as a percent of the control. Determined by multiplying the average SDW by the average SDW of the non-inoulcated control and multiplied by 100.

[2] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 3.5. Effect of growing temperature on the disease severity (DS) of plants infected with *Aphanomyces euteiches* root rot.

Experiment 1			Experiment 2		
Temp (°C)	DS[1]		Temp (°C)	DS	
16	1.2	c[2]	16	1.3	c
20	2.1	b	20	2.4	a
24	2.1	b	24	0.8	d
28	3.0	a	28	2.1	b
LSD	0.264		LSD	0.310	

[1] Average disease severity of 8 plants/pot, 3 replications.

[2] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 3.6. Effect of growing temperature on the root dry weight (RDW) of plants infected with *Aphanomyces euteiches* root rot.

	Experiment 1			Experiment 2			
Temp (°C)	RDW[1] (g)	RDW[2] (%)		Temp (°C)	RDW (g)	RDW (%)	
16	2.56	85.3	b[3]	16	1.99	72.6	b
20	1.68	77.8	c	20	1.87	85.4	b
24	3.17	74.8	a	24	2.53	101.0	a
28	1.67	81.9	c	28	1.37	92.6	c
LSD	0.297			LSD	0.203		

[1] Average root dry weight of 8 plants/pot, 3 replications.

[2] Average root dry weight as a percent of the control. Determined by dividing the average RDW by the average RDWof the non-inoculated control grown at the same temperature and multiplied by 100.

[3] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 3.7. Effect of growing temperature on the shoot dry weight (SDW) of plants infected with *Aphanomyces euteiches* root rot.

	Experiment 1			Experiment 2			
Temp (°C)	SDW[1] (g)	SDW[2] (%)		Temp (°C)	SDW (g)	SDW (%)	
16	3.40	95.0	a[3]	16	3.40	97.7	a
20	3.27	91.3	a	20	3.19	97.3	b
24	2.71	95.1	b	24	3.07	98.0	b
28	2.69	89.1	b	28	2.69	96.1	c
LSD	0.156			LSD	0.158		

[1] Average shoot dry weight of 8 plants/pot, 3 replications.

[2] Average shoot dry weight as a percent of the control. Determined by dividing the average SDW by the average SDW of the non-inoculated control grown at the same temperature and multiplied by 100.

[3] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

exhibited virulence differences on the eight lentil varieties tested. Isolates 25 and 26 had the same pattern of virulence on all hosts. The only isolate that was virulent on all lentil varieties was Isolate 22. French was the only variety that exhibited susceptibility to all seven *A. euteiches* isolates. Prior research on the effect of *A. euteiches* on lentils is limited if not non-existent. Because this was only a pathogenicity study, future research should focus on the full extent of *A. euteiches* impact on lentils (disease severity, host specialization). Lentils should also be investigated as another host to be used to resolve the confusion which already exists in the intraspecific taxonomy of *A. euteiches*, as noted above by Holub (1991b).

3.5 Conclusions

Strains of *A. euteiches* were isolated from approximately 20 percent of the fields surveyed in Manitoba. These fields ranged from Morden to Russell, Manitoba. This suggests that *A. euteiches* is more extensively spread in Manitoba than previously reported. With limited control measures available, one would recommend that the incidence of *A. euteiches* be closely monitored in order to limit the development of potentially devastating levels of the pathogen, which would seriously limit pea production in Manitoba.

Differences existed among the seven *A. euteiches* isolates ability to cause disease. One isolate was found to be significantly ($p=0.05$) more aggressive than the others, exhibiting the highest disease severity and lowest root and shoot dry weights under a controlled environment. However, plants inoculated with one of the seven *A. euteiches*

Table 3.8. Host-range of seven *Aphanomyces euteiches* isolates on six leguminous species.

		Isolates						
Host	**Variety**	**15**	**22**	**24**	**25**	**26**	**27**	**41**
Pea	AC Tamor	S[1]	S	S	S	S	S	S
Pea	Carneval	S	S	S	S	S	S	S
Pea	Majoret	S	S	S	S	S	S	S
Pea	Trapper	S	S	S	S	S	S	S
Lentil	CDC Glamis	S	S	R	R	R	S	S
Lentil	CDC Robin	S	S	S	S	S	S	R
Lentil	Crimson	R[2]	S	S	S	S	S	R
Lentil	Eston	R	S	R	S	S	S	R
Lentil	French	S	S	S	S	S	S	S
Lentil	Indianhead	R	S	S	S	S	R	S
Lentil	Laird	R	S	S	S	S	S	R
Lentil	Richlea	S	S	R	R	R	R	R
Chickpea	CDC Desiray	R	R	R	R	R	R	R
Chickpea	CDC Yuma	R	R	R	R	R	R	R
Bean	AC Black Diamond	R	R	R	R	R	R	R
Bean	CDC Pintium	R	R	R	R	R	R	R
Bean	AC Scarlet	R	R	R	R	R	R	R
Bean	Envoy	R	R	R	R	R	R	R
Bean	Navigator	R	R	R	R	R	R	R
Bean	Pintoba	R	R	R	R	R	R	R
Alfalfa	OAC Minto	R	S	S	R	R	S	R
Soybean	Alta	R	R	R	R	R	R	R

[1] S=Susceptible to *A. euteiches* isolate.

[2] R=Resistant to *A. euteiches* isolate.

isolates all had higher disease severity and lower root and shoot dry weights than the non-treated control. This is similar to reports by Grau *et al.* (1991), who found considerable variability in the pathogenicity of *A. euteiches*. Temperatures of 16-28°C were favourable for infection and subsequent disease development by the pathogen. Cool temperatures and moist soil favour infection, while warm temperatures are more conducive to disease development (Pfender, 1984).

The seven *A. euteiches* isolates exhibited host specificity among the crops and varieties tested. Isolates were pathogenic to pea, lentil and alfalfa varieties but were not pathogenic on bean, chickpea and soybean. This is consistent with past literature reported on the host-range of *A. euteiches*. We also know from previous reports by Lamari and Bernier (1985) that faba bean is susceptible to this pathogen. Caution should be taken when growing peas, lentils, alfalfa and in some cases faba bean. If proper rotations are not implemented this disease can develop into a serious problem as a result of the pathogen's ability to survive for long periods (>10 years) and the difficulties associated with controlling it (Pfender, 1984).

Since there are strains of *A. euteiches* that infect bean (*Phaseolus vulgaris* L.) (Pfender and Hagedorn, 1982), bean growers should pay close attention to the incidence of root rot in their fields. Low lying moist areas conducive to *A. euteiches* root rot should be monitored frequently. Field surveys should be conducted to specifically screen for the presence of *A. euteiches* and not just for pathogens which may be easier to isolate, such as *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp.

Chapter 4.0

The Effect of Seed Applied Fungicidal and Biological Treatments on the Control of *Aphanomyces euteiches* Drechs. Root Rot of Field Pea (*Pisum sativum* L.)

4.1 Abstract

Seed treatments (fungicide, biological agent or both), effective for the control of *Aphanomyces euteiches* root rot of field pea (*Pisum sativum* L.), would provide an alternative to crop rotation and avoidance; methods currently used for control. To determine the effectiveness of seed applied fungicide and biological treatments, experiments testing seven fungicides (Aliette, Apron, Crown, Ridomil, Ronilan, Thiram, and Vitaflo-280) and two biological agents, ACM941 (ATCC 74447) and AR101, were conducted in 1999 and 2000. Both biological agents are strains of *Gliocladium roseum* Bainier. Three combination treatments were also tested, consisting of both a fungicide and a biological agent (ACM941+Aliette, ACM941+Apron and ACM941+Vitaflo-280). Disease symptoms were evaluated from field plots at three locations. A natural source of inoculum was relied upon at each location. Emergence, disease severity, root and shoot dry weights and yield were measured to determine the effect of each treatment on Aphanomyces root rot. Percent emergence was significantly different ($p=0.05$) among treatments at each site. Although, the overall effect of treatments on emergence varied between sites. None of the thirteen treatments tested significantly controlled *Aphanomyces euteiches* root rot of field pea. However, when test sites were compared, differences were observed within and between sites. Average disease severity and yield ranged from 0.3 to 3.9 and 467g to 1227g, respectively. These differences may have resulted from variable environmental conditions (temperature, moisture and soil type)

between sites and years, and/or the relative aggressiveness of the *A. euteiches* isolates present. An increase in inoculum pressure, resulting from growing the same crop in consecutive years could also explain the increase in disease severity. Seed treatment efficacy could also be hindered by *A. euteiches* ability to infect all underground plant tissue throughout the growing season.

4.2 Introduction

In 2002, Manitoba produced 13 percent of the Western Canadian dry pea crop. The area covered 200,000 acres and produced 6.5 million bushels (Manitoba Agriculture and Food, 2003). The current figures are slightly lower than the record highs in 1998 when 255,000 acres and 8.3 million bushels were reported. Manitoba markets primarily yellow peas for food and livestock feed along with smaller amounts of green peas (Anonymous, 2003). Manitoba Agriculture and Food (2003) reported average prices of $4.87 and $5.50 per bushel for the 2001 and 2002 crops, respectively. Annual cash receipts for the sector have fluctuated over the past 10 years, increasing from $12.2 million in 1993 to $27.7 million in 1998, decreasing to $15 million in 2000 and then rising to record levels of $30 million in 2002. In 2001, Manitoba's contribution to Canada's annual dry pea receipts was 6.7 percent. This is down significantly from the 23.3 percent Manitoba contributed in 1992.

The soil-borne oomycete pathogen *Aphanomyces euteiches* Drechs., is common in many regions of the United States, Canada, Europe, Asia and Australia (Papavizas and Ayers, 1974). Due to its importance as a root-infecting pathogen of legumes, particularly pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.), snap bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba*) and red clover (*Trifolium pratense* L.), it has been widely studied (Delwiche *et al.*, 1987; Grau *et al.*, 1991; Munkvold and Carlton, 1995; Papavizas and Ayers, 1974; Lamari and Bernier, 1985; Pfender *et al.*, 1984). Pea appears to be the crop most significantly impacted by Aphanomyces root rot, with reports of complete crop loss (Pfender, 1984; Papavizas and Ayers, 1974).

Presently, there are no commercially available root rot resistant pea cultivars or registered biological or chemical controls (Gritton *et al.*, 1995). Although many control measures have been tested (Papavizas and Ayers, 1974), the only efficient way to control this disease is to avoid planting peas in infested fields and to ensure that there is an appropriate time interval between successive pea crops (Carrouée *et al.*, 1995).

The objective of this study was to evaluate the effectiveness of fungicide and biological agents seed treatments on the suppression of Aphanomyces root rot of pea, with the goal of developing an integrated control strategy.

4.3 Materials and Methods

4.3.1 Site Location and Experimental Design

Trials were conducted at three sites in 1999 and 2000. All sites used in this study had a history of Aphanomyces root rot. Therefore, a natural source of inoculum was relied upon at all three sites. The first site was located in Winnipeg, Manitoba, at the University of Manitoba campus farm. This field has a known *Aphanomyces* infestation, dating as far back as 70 years. This site, referred to as "Wpg", was used in both years of the study. This was located on a clay-loam soil with lentil residue. In 1999, the second site, referred to as "Penner", was located on a commercial farm in the Municipality of Roland, north of Morden, Manitoba. This was located on a sandy loam soil with pea residue. In 2000, the second site was located at Agriculture and Agri-Food Canada's Morden Research Station (MRS) located in Morden, Manitoba and will be referred to as "MRS". This was located on a clay loam soil with wheat residue.

Throughout the study, plots consisted of four 3-m-long rows with 12 cm spacing. The variety of pea used was Carneval. Each trial was conducted as a randomized complete block design (RCBD) with four replications. Treatments included seven fungicides and two biological control agents. The seven fungicides tested were Aliette, Apron, Crown, Ridomil, Ronilan, Thiram, and Vitaflo-280. The two biocontrol agents were strains of *Gliocladium roseum* Bainier, ACM941 and AR101, provided by Dr. A. Xue, Agriculture and Agri-Food Cananda, Morden, MB (Xue, 2002). Three treatments included a combination of a fungicide and a biocontrol agent: ACM941+Aliette, ACM941+Apron and ACM941+Vitaflo-280. The complete list of treatments is presented in Table 4.1.

4.3.2 Seed Treatment

Prior to seeding each year, 581 g seed lots of Carneval were treated and used in each of the two locations. Using a seed counter, packages of 288 treated seeds were prepared. Each package had an average weight of 63 g.

Spore suspensions for the biological seed treatments, ACM941 and AR101, were prepared by growing each isolate on potato dextrose agar in 9-cm Petri plates. Once growth was established, 5-10 ml of a 0.5% Tween 20 solution was added to each plate. The cultures were harvested by gently scraping the spores loose from the surface of the media using a sterile microscope slide. Once dislodged, the spores were passed through two layers of cheesecloth into a beaker.

Table 4.1. Seed applied fungicidal and biological agent rates used in the 1999 and 2000 field trial studies of *Aphanomyces euteiches* root rot.

Treatment (Trade name)	**Active Ingredient**	**Rate (per Kg of seed)**
ACM941 10^8 spores ml-1	*Gliocladium roseum*	10.0 ml
AR101 10^8 spores ml-1	*Gliocladium roseum*	10.0 ml
ACM941 + Aliette 80W	*Gliocladium roseum* and Fosetyl Al	10.0 ml + 2.5 g ai
ACM941 + Apron FL	*Gliocladium roseum* and Metalaxyl[s]	10.0 ml + 0.32 ml (+ 4.68 ml water)
ACM941 + Vitaflo-280	*Gliocladium roseum* and Carbathiin[s] and Thriam[c]	10.0 ml + 2.6 ml
Aliette 80W	Fosetyl Al	2.5 g ai
Apron FL	Metalaxyl	0.32 ml in 4.68 ml water
Apron FL	Metalaxyl	1.0 ml in 4.0 ml water
Crown	Carbathiin and Thiabendazole[s]	6.0 ml
Ridomil	Chlorothalonil[c] and Metalaxyl	3.0 g ai
Ronilan 50DF	Vinclozolin[c]	3.0 g ai
Thiram 75WP	Thiram	1.0 g ai
Vitaflo-280	Carbathiin and Thiram	2.6 ml

s= systemic fungicide
c= contact fungicide

Spore concentrations were determined using a haemocytometer and adjusted to 10^8 spore ml^{-1} by adding sterile distilled water amended with Tween 20. Spore suspensions were poured into bags containing pea seeds at the rates listed in Table 4.1. Each bag was shaken to ensure that all seeds were evenly coated with spores. Bags were shaken every 15 minutes for 1 hour and left open to dry overnight. In cases where both a fungicide and biological agent were used, the fungicide was applied after the biological agent.

In a fume-hood, fungicide treatments were applied directly to the seed in pretreated 7 kg plastic bags. Each treatment was added at the rate listed in Table 4.1. For treatments where water was required, the fungicide was first mixed with water then applied to the seed. Bags were shaken in order to ensure all seeds were evenly coated and left open to dry overnight.

4.3.3 Plot Information

Plots in Wpg were seeded on May 18 and 9, in 1999 and 2000 respectively. This site received approximately 16 kg/ha of 12-52-0 (N-P-K), applied directly with the seed in 1999 and 2000. Plots at the Penner site were seeded on April 29, 1999. The MRC plots were seeded on May 8, 2000 and received 9 kg/ha of P_2O_5 in the fall of 1999.

Herbicides were applied to plots to remove unwanted grassy and broadleaf weeds. In 1999, the entire Wpg site was sprayed with Roundup™ (775 g glyphosate/ha) prior to seeding to control an infestation of Canada thistle at the site; plots were subsequently hand-weeded throughout the growing season. The Penner site was sprayed with Basagran (310 g/ha) on June 25, 1999 and Poast Ultra (77 g/ha) + Merge (155 g/ha) on

July 7, 1999. In 2000, the entire Wpg site was sprayed with Roundup (775 g glyphosate/ha) on May 16 to control thistles at the site, Poast (426 g/ha) on June 6 and subsequently hand-weeded. Plots at MRS were sprayed with Basagran (350 g/ha) + Assist oil (155 g/ha) on June 22, 2000, Poast Ultra (100 g/ha) + Merge (155g/ha) on June 27, 2000 and then again with Basagran (350 g/ha) + Assist oil (155 g/ha) on July 12, 2000.

Disease severity (DS) was recorded twice during the growing season at all sites, when possible. In 1999, the first DS rating was recorded on June 29 (42 days after planting) at Wpg and June 15 (47 days after planting) at Penner. The second DS rating was recorded on July 27 (70 and 89 days after planting, respectively) at both Wpg and Penner. In 2000, the first DS rating was evaluated on July 4 (56 days after planting) at Wpg and July 7 (60 days after planting) at MRC and the second DS rating was evaluated on July 26 (79 days after planting) at MRC. Due to excessive moisture during the spring of 2000, first DS ratings were delayed until the beginning of July and a second rating was unattainable at Wpg due to a complete loss of the plots.

Plots were harvested August 13, 1999 (87 days after planting) in Wpg using a Hege combine (Hege Maschinen GmbH, Waldenburg, Germany). Due to excessive moisture in the spring of 2000, plants succumbed to severe disease pressure and no material was harvested from the Wpg site. Penner and MRC plots were harvested August 15, 1999 (108 days after planting) and August 14, 2000 (98 days after planting), respectively, using a Wintersteiger Elite small plot combine (Wintersteiger, Laval, Quebec).

Rainfall and temperature data for each site is shown in Appendix 6.

4.3.4 Disease Assessment

Measurements to determine the effect of each treatment on the control of *A. euteiches* in field pea included emergence counts, disease severity (DS), root and shoot dry weight (DW), yield, and total seed weight (TSW). Disease severity was determined twice during the growing season using Sherwood's (1958) 0-4 system of rating (Figure 3.1 a-f). The disease classes employed were as follows: 0 - Healthy roots; 1 - Roots with a few water-soaked, light brown areas; 2 - Roots water-soaked, light brown areas confluent and more extensive but not involving the entire root system; 3 - Water-soaked and browning involving all roots and epicotyl (stem above seed piece), tissue soft but not collapsed, epicotyl not markedly shriveled; 4 - Water-soaked, browning and decay involving all roots and epicotyl, cortex easily sloughed off, epicotyl shriveled or rotted.

Disease severity was determined by uprooting 1m length sections from either of the outside rows of each plot. Plant roots were rinsed thoroughly of any debris and visually rated for symptoms present on the epicotyl and root system. Root and shoot dry weights were determined by dividing the plant into sections at the point along the epicotyl where chlorophyll pigmentation began and ended. These sections were then placed in paper bags and dried for 48 hours at 80°C before being weighed.

Yield was determined by harvesting the remaining plant stand of each plot. Seed was placed on a heated bed and dried for approximately one week. Once dry, the seed was cleaned and weighed.

4.4 Statistical Analysis

Statistical analysis was conducted using SAS (Version 6.12 or 7.00 ©1998, SAS Institute Inc. Cary, NC, USA). Locations were analyzed separately because site, as a major factor, was significant at p=0.05 when analyzed together.

4.5 Results and Discussion

Disease development varied with year and location but symptoms were present in each trial (Figure 4.1). In 2000, the Winnipeg trial was lost when all plants succumbed to Aphanomyces root rot before reaching the mature dry seed stage. For each experiment, root tissue was examined under a compound microscope (Zeiss, Oberkuchen, Germany) for the presence of oospores characteristic of *A. euteiches* (Figure 4.2 a-c). Pure cultures of the pathogen were isolated and healthy pea plants were inoculated, as described in Chapter 3. Pathogen identification and isolations were performed on up-rooted plants exhibiting symptoms typical of *A. euteiches*. Koch's Postulates (1882) were necessary to ensure that the symptoms observed were consistent with those caused by *A. euteiches* and not secondary invaders or saprophytes colonizing tissue that died from other causes. *Aphanomyces euteiches* is one of many pathogens included in Canada's pea root rot complex (PRRC) (Tu, 1986 & 1991). The other pathogens included *Alternaria alternata*, *Fusarium oxysporum* f. sp. *pisi*., *F. solani* f. sp. *pisi*., *Mycophaerella pinodes*, *Pythium* spp., *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. Determining whether *A. euteiches* had infected such roots was therefore dependant upon microscopic examination for the presence of the characteristic large, thick-walled oospores (Walker, 1952).

A)

B)

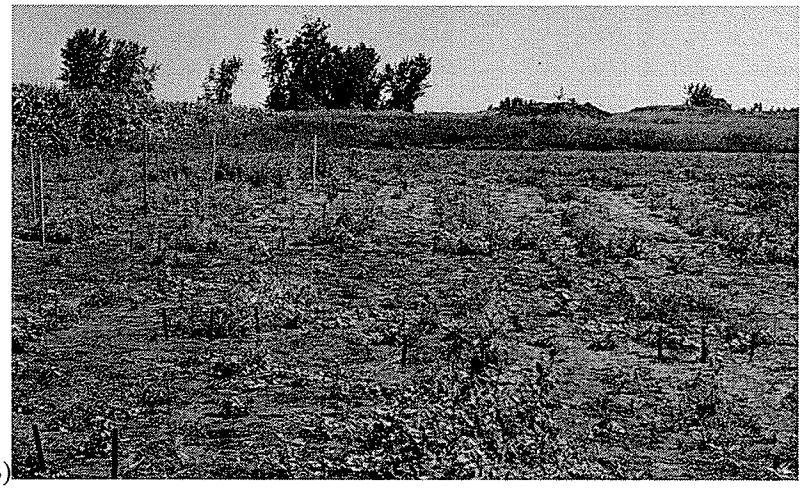

Figure 4.1. Field trial testing the effectiveness of fungicide and biological seed treatments on the control of *A. euteiches* root rot, located at Penner in 1999. A) Early established pea plots. B) Pea plots severely affected by Aphanomyces root rot.

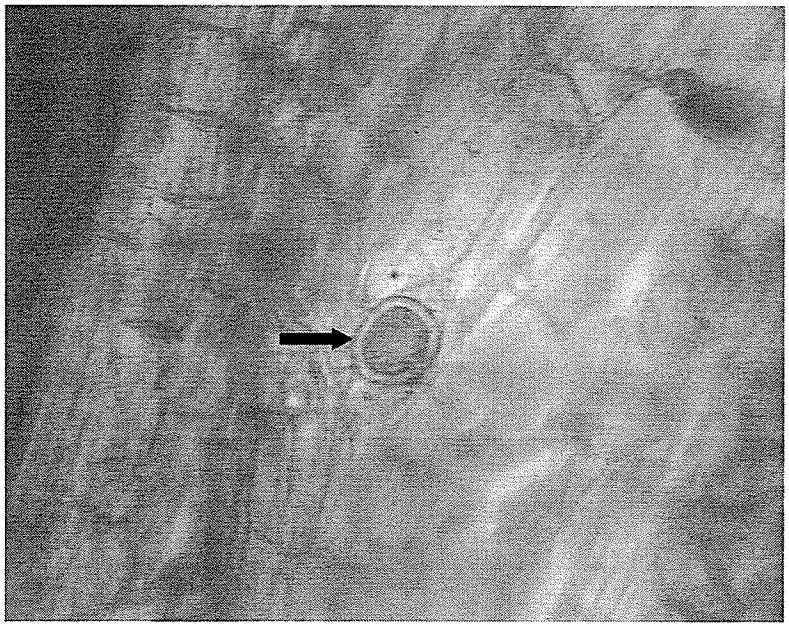

Figure 4.2. Microscopic observation of *Aphanomyces euteiches*. Oospore embedded in the root cortical tissue of field pea, 100x.

The fungicides tested in this experiment control several seed rots and seedling blights caused by *Pythium* spp., *Botrytis* spp, *Fusarium* spp, and *Rhizoctonia solani*, early and late blight of potato, Sclerotinia stem rot, downey-mildew of pea, and seedborne seedling blight caused by *Ascochyta*. Apron FL, Thiram and Vitaflo-280 are listed in the Guide to Crop Protection (2001) as seed applied fungicides registered for the control of seed and seedling rots/blights in pea. Apron FL and Ridomil are specifically registered for the control of oomycetes, to which A. *euteiches* belongs.

4.5.1 Emergence

Treatments had a significant (p=0.05) effect on emergence at each site in both years (Tables 4.2-5 and Figure 4.3). This may suggest a certain level of Aphanomyces suppression or seed toxicity resulting from the seed applied treatments. At Wpg in 1999, Thiram and Apron 0.3 FL were the only treatments significantly better than the non-treated control. Ronilan, ACM941+Aliette and Aliette were the only treatments with lower (but not significant) emergence counts than the non-treated control. At Penner in 1999, Ridomil and ACM941+Apron FL were significantly better than the non-treated control. The only treatments with lower (but not significant) emergence counts than the non-treated control were Ronilan, Crown and AR101. At both Wpg and MRC in 2000, no treatments were significantly better than the non-treated control. However, the non-treated control was significantly better than Vitaflo-280 and Apron 0.3 FL at WPG and Vitaflo-280 at MRC. Although significant differences separated the treatments at each site, the overall effect that individual treatments had on emergence varied between sites.

Table 4.2. Effect of thirteen seed treatments on Aphanomyces root rot of pea, Winnipeg, 1999.

Treatment	Emergence[1] (% control)	DS1[2] 0-4 scale	DS2[2] 0-4 scale	RootDW1[3] (g)	RootDW2[3] (g)	ShootDW1[4] (g)	ShootDW2[4] (g)	Yield (g)
ACM941+Vitaflo	103.8 ab[5]	1.7 a	4.0 a	3.48 a	1.79 ab	43.9 ab	287.7 ab	1066.8 ab
Apron 0.3 FL	106.3 a	1.7 a	4.0 a	3.88 a	2.82 a	46.2 ab	362.2 a	1128.3 ab
Aliette 80W	98.2 bc	1.6 a	4.0 a	3.02 a	1.77 ab	37.5 b	260.9 ab	1197.3 ab
ACM941	105.4 ab	1.6 a	4.0 a	3.47 a	1.98 ab	41.3 ab	326.0 ab	1186.4 ab
Non-Treated Control	100.0 bc	1.6 a	4.0 a	3.49 a	2.31 ab	42.9 ab	295.3 ab	1183.8 ab
ACM941+ Apron	103.7 ab	1.5 a	4.0 a	3.19 a	2.16 ab	42.6 ab	281.6 ab	1104.5 ab
Apron 1.0 FL	103.8 ab	1.5 a	4.0 a	3.22 a	2.48 ab	42.6 ab	350.9 a	1192.6 ab
Thiram 75WP	107.9 a	1.5 a	4.0 a	3.07 a	1.97 ab	45.6 ab	294.3 ab	1268.4 a
Crown	97.4 d	1.4 a	4.0 a	3.59 a	1.55 b	45.7 ab	222.4 b	934.8 b
ACM941+ Aliette	99.4 bc	1.3 a	4.0 a	3.24 a	2.33 ab	45.7 ab	328.8 ab	1189.4 ab
Ronilan 50DF	99.8 bc	1.2 a	4.0 a	3.13 a	1.97 ab	41.8 ab	270.6 ab	1180.6 ab
AR101	104.3 ab	1.2 a	4.0 a	3.64 a	2.05 ab	51.0 a	303.8 ab	1103.0 ab
Ridomil	105.9 ab	1.1 a	4.0 a	3.62 a	2.03 ab	44.2 ab	253.2 ab	956.5 b
Vitaflo-280	104.2 ab	1.0 a	4.0 a	3.63 a	1.78 ab	48.3 a	285.9 ab	1239.4 a
LSD	6.00	0.8	0.0	0.90	1.07	9.96	124.1	270

[1] Emergence data represent the average number of seedlings/3m linear section (3 replications) divided by the average non-treated control and multiplied by 100.

[2] Disease severity for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[3] Root Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[4] Shoot Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[5] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 4.3. Effect of thirteen seed treatments on Aphanomyces root rot of pea, "Penner", 1999.

Treatment	Emergence[1] (% control)	DS1[2] 0-4 scale	DS2[2] 0-4 scale	RootDW1[3] (g)	RootDW2[3] (g)	ShootDW1[4] (g)	ShootDW2[4] (g)	Yield (g)
Vitaflo-280	105.7 ab[5]	4.0 a	4.0 a	1.48 a	1.56 a	10.2 a	138.0 a	386.9 ab
ACM941+ Vitaflo	104.7 abc	4.0 a	4.0 a	1.52 a	1.34 a	11.3 a	121.8 a	255.4 b
Apron 0.3 FL	105.7 ab	3.9 a	4.0 a	2.18 a	1.76 a	14.1 a	129.8 a	397.7 ab
ACM941	102.8 abc	3.9 a	4.0 a	1.81 a	1.49 a	13.5 a	104.4 a	348.8 ab
Aliette 80W	102.5 abc	3.9 a	4.0 a	1.59 a	2.62 a	13.3 a	170.7 a	683.8 a
ACM941+ Aliette	104.5 abc	3.8 a	4.0 a	1.85 a	1.56 a	15.3 a	110.6 a	487.2 ab
Crown	99.67 bc	3.8 a	4.0 a	2.16 a	1.56 a	13.9 a	143.7 a	475.4 ab
ACM941+ Apron	106.6 a	3.8 a	4.0 a	1.94 a	1.47 a	15.1 a	112.1 a	442.9 ab
Ridomil	108.6 a	3.8 a	4.0 a	1.59 a	1.91 a	11.2 a	163.9 a	545.8 ab
Non-Treated Control	100.0 bc	3.8 a	4.0 a	1.84 a	2.42 a	15.1 a	137.8 a	636.0 a
Apron 1.0 FL	102.8 abc	3.7 a	4.0 a	1.79 a	2.15 a	13.5 a	168.2 a	608.6 ab
Thiram 75WP	106.3 ab	3.7 a	4.0 a	2.11 a	2.41 a	14.5 a	145.4 a	578.7 ab
AR101	98.78 c	3.6 a	4.0 a	1.81 a	2.70 a	14.6 a	163.9 a	529.3 ab
Ronilan 50DF	99.67 c	3.5 a	4.0 a	1.84 a	1.75 a	15.4 a	110.2 a	626.3 ab
LSD	6.39	0.55	0.0	0.814	1.83	5.41	91.1	371.59

[1] Emergence data represent the average number of seedlings/3m linear section (3 replications) divided by the average of the non-treated control and multiplied by 100.

[2] Disease severity for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[3] Root Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[4] Shoot Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[5] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 4.4. Effect of thirteen seed treatments on Aphanomyces root rot of pea, Winnipeg, 2000.

Treatment	Emergence[1] (% control)	DS1[2] 0-4 scale	RootDW1[3] (g)	ShootDW1[4] (g)
AR101	106.7 a[5]	4.0 a	1.99 a	23.2 ab
Vitaflo-280	87.4 c	4.0 a	1.94 a	16.1 b
Apron 0.3 FL	90.3 c	4.0 a	1.76 a	20.8 ab
ACM941+ Apron	96.3 bc	4.0 ab	2.49 a	22.4 ab
Ridomil	100.7 ab	4.0 ab	2.30 a	23.7 ab
Ronilan 50DF	102.2 ab	4.0 ab	2.59 a	22.5 ab
Aliette 80W	92.6 bc	4.0 ab	1.75 a	20.0 ab
ACM941	93.7 bc	4.0 ab	2.11 a	22.6 ab
Thiram 75WP	97.4 ab	4.0 ab	2.13 a	19.7 ab
Apron 1.0 FL	92.6 c	3.9 ab	2.55 a	30.5 a
ACM941+ Vitaflo	96.8 bc	3.9 ab	2.05 a	18.9 ab
ACM941+ Aliette	91.8 c	3.9 ab	1.67 a	19.8 ab
Crown	62.6 d	3.9 ab	2.35 a	19.1 ab
Non-Treated Control	100.0 ab	3.9 b	1.92 a	20.2 ab
LSD	9.37	0.11	1.13	12.1

[1] Emergence data represent the average number of seedlings/3m linear section (3 replications) divided by the average non-treated control and multiplied by 100.

[2] Disease severity for the first (1) assessment date. Represents plants/1m linear section.

[3] Root Dry Weight for the first (1) assessment date. Represents plants/1m linear section.

[4] Shoot Dry Weight for the first (1) assessment date. Represents plants/1m linear section.

[5] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table 4.5. Effect of thirteen seed treatments on Aphanomyces root rot of pea, Morden Research Centre, 2000.

Treatment	Emergence[1] (% control)	DS1[2,6] 0-4 scale	DS2[2] 0-4 scale	RootDW1[3,6] (g)	RootDW2[3] (g)	ShootDW1[4,6] (g)	ShootDW2[4] (g)	Yield (g)
Thiram 75WP	97.3 abc[5]	0.6 a	1.9 ab	3.50 ab	2.15 a	115.6 a	292.0 a	1234.6 ab
Aliette 80W	100.7 a	0.5 ab	1.6 b	3.09 ab	2.43 a	113.8 a	300.6 a	1378.7 ab
Apron 0.3 FL	94.6 abc	0.4 abc	1.9 ab	2.47 ab	2.65 a	109.0 a	312.9 a	1462.2 a
Ronilan 50 DF	99.1 ab	0.4 abc	2.0 a	3.24 ab	2.05 a	120.0 a	270.9 a	1377.0 ab
ACM941+ Apron	94.3 abc	0.4 abc	1.8 ab	2.49 ab	1.85 a	124.4 a	284.2 a	1279.4 ab
Non-Treated Control	100.0 ab	0.3 abc	1.8 ab	2.51 ab	2.28 a	97.6 a	323.8 a	1240.4 ab
Crown	95.2 abc	0.3 abc	1.9 ab	3.34 ab	2.33 a	117.7 a	298.5 a	1322.1 ab
Vitaflo-280	90.8 c	0.3 abc	1.7 ab	3.82 ab	2.08 a	99.1 a	283.9 a	1309.4 ab
ACM941	97.2 abc	0.2 bc	1.8 ab	2.59 ab	2.15 a	107.3 a	283.7 a	1357.4 ab
Ridomil	99.3 ab	0.2 bc	1.9 ab	4.37 a	2.43 a	118.8 a	302.1 a	1153.8 b
Apron 1.0 FL	94.1 bc	0.2 bc	1.9 ab	3.28 ab	2.28 a	122.0 a	284.1 a	1308.7 ab
ACM941+ Aliette	94.2 abc	0.2 bc	1.8 ab	1.77 b	2.20 a	110.6 a	281.5 a	1252.3 ab
ACM941+ Vitaflo	96.1 abc	0.1 bc	1.9 ab	4.36 a	2.28 a	119.0 a	301.5 a	1346.7 ab
AR101	97.5 ab	0.1 bc	1.8 ab	3.32 ab	2.68 a	117.5 a	300.4 a	1384.3 ab
LSD	6.49	0.4	0.4	2.25	0.85	40.4	63.5	253.18

[1] Emergence data represent the average number of seedlings/3m linear section (3 replications) divided by by the average non-treated control and multiplied by 100.

[2] Disease severity for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[3] Root Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[4] Shoot Dry Weight for first (1) and second (2) assessment dates. Represents plants/1m linear section.

[5] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

[6] Values based on assessment of blocks 1 and 3 only.

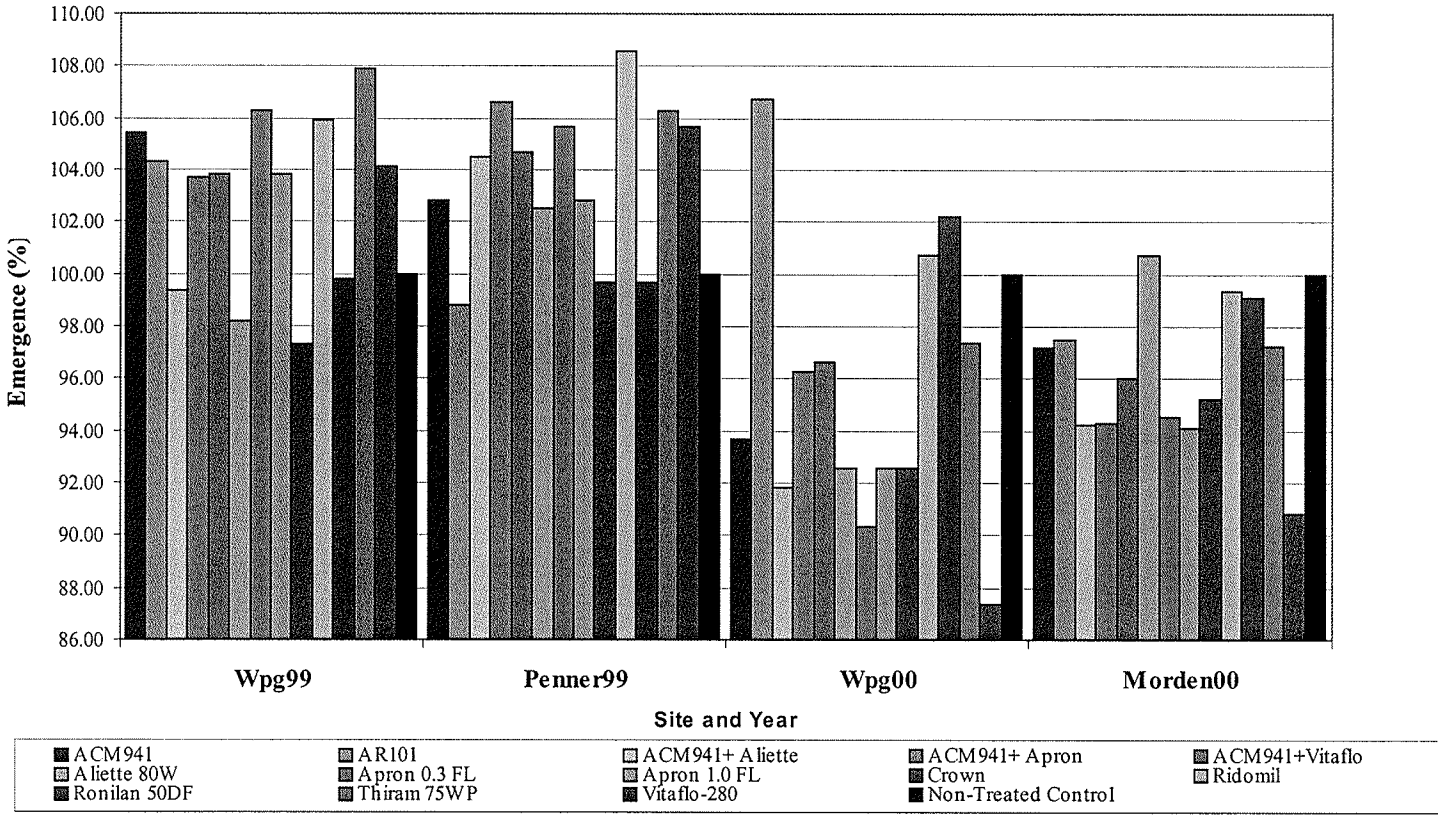

Figure 4.3. Effect of 13 seed treatments on the control of Aphanomyces at four field locations. Obtained from field trials conducted in 1999 and 2000. Bars represent emergence counts (% control).

Due to this variability, we can assume that the treatments provided little to no control of Aphanomyces and were not toxic to the seed at the concentrations used.

As the only fungicides registered for oomycete control, there was particular interest in how Ridomil and Apron preformed as compared to the non-treated control. In 1999, at both sites, percent emergence for Ridomil (105.9 % at Wpg and 108.6 % at Penner), ACM941+Apron (103.7% at Wpg and 106.6% at Penner) and both Apron treatments (Apron 0.3 FL: 106.3 % at Wpg and 105.7 % at Penner; and Apron 1.0 FL: 103.8 % at Wpg and 102.8 % at Penner) were higher than the non-treated control (Tables 4.2 and 4.3). Apron 0.3 FL at Wpg and Ridomil and ACM941+Apron at Penner were significantly better than the non-treated control. Similar results were found at Wpg in 2000, although the mean of Ridomil (100.7 %) was only slightly, but not significantly, higher than the non-treated control (Table 4.4). However, in 2000, percent emergence for ACM941+Apron (96.28% at Wpg and 94.31% at MRC) and both Apron treatments (Apron 0.3 FL: 90.33 % at Wpg and 94.56 % at MRC; and Apron 1.0 FL; 92.57 % at Wpg and 94.11 % at MRC) were lower than the non-treated control, at both sites (Tables 4.4 and 4.5). The non-treated control was significantly better than Apron 0.3 FL, at Wpg. Ridomil (99.33 %) also exhibited a lower, but not significantly, percent emergence than the non-treated control, at MRC in 2000. The latter of these findings supports observations made by Pfender *et al.* (1984), who reported *A. euteiches* to be insensitive to metalaxyl, the active ingredient in Ridomil and Apron FL. Therefore, any effect that these chemicals had on emergence could be due to their impact on other pathogens within the pea root rot complex. This applies specifically to Apron FL, which is registered for the control of oomycetes, such as *Pythium* spp.

When assessing seed applied treatments, emergence may be the only parameter to consider due to the short duration of control provided by seed-applied treatments. Apron FL, Crown, Ridomil and Vitaflo-280 are fungicides with systemic activity, which enables the chemical to be taken-up by the plant as it develops providing protection against pathogens throughout the plant. For instance, Apron FL is registered for seed rots and seedling blights caused by *Pythium* spp., where it may provide 2 to 3 weeks of protection (Guide to Crop Protection, 2001). Therefore, even though a fungicide is systemic, the ability to provide long-term protection may be somewhat limited. Emergence values can help to differentiate treatments by their relative ability to suppress *A. euteiches* root rot in pea during this brief window of opportunity. A plant's ability to germinate and establish itself is especially important when exposed to a root rot pathogen such as *A. euteiches*. Aphanomyces can infect throughout the growing season, attacking all underground portions of the pea plant (Jones and Drechsler, 1925). Xue (2002) stated that the ability of the biological control agent ACM941 to survive and propagate within the rhizosphere gives it the potential to provide effective and long-lasting plant protection. This is in contrast with chemical fungicides that have a limited period of effectiveness. Results of this experiment both agreed and disagreed with Xue's (2002) findings, in that, we did not find that the chemical fungicide or biological treatments tested provided significant control of Aphanomyces root rot over a long period of time. However, given *A. euteiches* ability to infect throughout the growing season, one can assume that seed-applied treatments would not provide the long-term protection necessary to control this root rot pathogen.

4.5.2 Disease Measurements

Treatments did not have a significant effect on disease severity, dry root and shoot weights and yield, with the exception of DS1 at MRC in 2000 (Tables 4.2-5). This suggests that the 13 seed-applied treatments did not effectively control this disease during the growing season.

Disease Severity

Although treatments were found to be non-significant, differences were observed when average disease severity values were compared between sites and years. The range in average disease severity, from DS1 values, varied considerably between sites and years. In 1999, average disease severity ranged from 1.4 at Wpg to 3.8 at Penner and in 2000, average disease severity ranged from 0.3 at MRS to 3.9 at Wpg (Tables 4.2-5). These differences could be due to varying environmental conditions (temperature, moisture and soil type), and/or differences in initial inoculum levels at the different sites. This is evident at Wpg where average disease severity ranged from 1.4 to 3.9 in 1999 and 2000, respectively. We can assume that these differences were primarily a result of environmental conditions since the same variety of pea was planted in the same field both years. However, one can also argue that this range in average disease severity resulted from an increase in the level of *A. euteiches* inoculum as a result of successive plantings of susceptible hosts. This is in agreement with the report of Jones and Linford (1925), who found that the extent of infestation in a field was closely related to the number and frequency of previous pea crops. Tu (1987) also stated that the incidence and severity of root rots are related to the density of inocula in the soil. More likely, a combination of

environmental conditions and consecutive pea cropping was responsible for the differences in disease severity averages. Contrasts between sites could be due to inoculum levels and/or differences in the relative aggressiveness of the *A. euteiches* isolates present. Populations of *A. euteiches* isolated from pea can be pathogenically, genotypically, and geographically variable (Malvick and Percich, 1998b).

Yield

Yield was highly variable between sites. It ranged from 934.8 to 1268 g at Wpg in 1999, 255.4 to 683.8 g at Penner in 1999, and 1153.8 to 1462.2 g at MRC in 2000 (Tables 4.2-5). The non-treated control (636.0) was significantly different (p=0.05) than all treatments, except Aliette 80W at Penner in 1999. Growing conditions in 1999 and 2000 were conducive to severe root rot due to excessive moisture at the beginning of each season. This was especially evident at Wpg in 2000, where plots were completely lost prior to maturity. This supports findings by Pfender (1984) and Papavizas and Ayers (1974), who reported that pea fields with 100% yield loss were common. Excessive moisture may have enhanced *A. euteiches* ability to infect and hindered the effectiveness of the treatments. However, one would expect disease pressure to be more severe the second year, following planting of susceptible hosts. Tu (1987) reported that fields in which root rots have been severe are likely to have the same problem the next time a susceptible cultivar is planted. Reiling *et al.* (1959) found that as the degree of root rot increased, vine weight and pod yield decreased.

Although yield varied between sites, treatments did not significantly affect yield. The lack of control observed in this study could be explained by favourable moisture

conditions for pathogen development, in addition to *A. euteiches* ability to attack throughout the growing season.

4.6 Conclusions

Differences in emergence counts suggest that some level of control was provided against Aphanomyces root rot earlier in the season. As the season progressed, fungicide efficacy decreased and severe root rot developed suggesting that pathogen control was lost. Fungicide seed treatments were not effective to control Aphanomyces root rot when inoculum pressure is high and moisture favourable. Burke *et al.* (1969) found that localized treatments for control of Aphanomyces were not encouraged due to the pathogen's ability to infect along the root system, as well as move up within the stem. Older plants are not resistant to the fungus. Factors contributing to the overall success of Aphanomyces include its long persistence in infested soil, rapid build-up of inoculum, ability to reproduce sexually via oospores and asexually via zoospores, and the lack of effective control measures. The pathogen's ability to infect throughout the growing season adds to its overall success as a root rot pathogen.

Pfender (1984) stated that this disease may be managed but not controlled; at least with current control measures. Results from this study suggest that this statement is true. Some recommendations to decrease the risk of Aphanomyces root rot are to implement lengthy crop rotations (up to 6 years) and/or avoid fields heavily infested with the pathogen. Therefore, the most effective way to prevent losses is by knowing the current root rot potential of a field, either through field history or assessing infestation levels prior to seeding.

Chapter 5.0

General Discussion

Survey results suggested that *A. euteiches* is more extensively spread than previously recorded. The presence of *A. eutieches* ranged a distance of 400 km, from Morden to Russell, Manitoba. Previous reports suggested that Aphanomyces root rot was restricted to the Red River Valley (Lamari and Bernier 1985; Mathur et al., 1998). Knowledge of Aphanomyces incidence is very important since proper crop rotation and/or avoidance are the only effective control measures to date.

Pathogenicity differences were observed between the seven *A. euteiches* isolates. This is consistent with previous knowledge of the pathogen. Isolates of the pathogen vary with respect to the degree of virulence expressed on various pea lines (Pfender, 1984). Temperatures of 16-28°C were favourable for infection and subsequent disease development by *A. euteiches*. Burke *et al.* (1969) reported temperatures of 16-28°C to be favourable for pea germination and growth, as well as favourable for infection and subsequent disease development by *A. euteiches*.

Host-range studies indicated that pea, lentil and alfalfa seedlings were susceptible to the seven *A. euteiches* isolates, whereas chickpea, bean, and soybean were resistant. This suggests that crop rotations which exclude pea, lentil and alfalfa, and in some cases faba bean (Lamari and Bernier, 1985), may be implemented in order to minimize the build-up of inoculum in these fields. However, rotations should primarily consist of non-leguminous crops (Sherwood and Hagedorn, 1962).

Seed treatment trials demonstrated that fungicide and biological control treatments had little effect on the control of *A. euteiches* root rot of pea. Although none

of the fungicides tested were registered for control of *A. euteiches* in pea, this study does offer a detailed look at the response of the pathogen to a broad array of treatments. The results of the present study agree with Malvick and Percich (1998b), who reported that root rot diseases caused by *A. euteiches* can be reduced through rotation and/or avoidance of highly infested fields but cannot be controlled commercially with chemicals or host resistance.

In 2000, approximately 1.2 million ha of field pea (*Pisum sativum* L.) were grown, classifying it as the fourth major crop by acreage of production after wheat, canola, and barley in Canada (Statistics Canada, 2000). Production area is expected to increase to more than 2 million ha by 2005 (Xue, 2002). In the USA, Aphanomyces root rot has remained a destructive disease of pea for over 70 years (Jones and Drechsler, 1925) due to ineffective disease control methods. Crop rotation, host resistance, fungicides and biological control agents have all been investigated to manage this disease (Pfender, 1984; Papavizas and Ayers, 1974; Xue, 2002). These tactics have shown promise, but have not yet provided an adequate level of control for commercial production.

Although the isolates obtained from this study were not pathogenic on bean, close attention should be paid to *A. euteiches* f. sp. *phaseoli*, the species with selective pathogenicity to bean (Pfender and Hagedorn, 1982). Manitoba is the largest producer of dry beans in Canada, producing 56.8 percent of the Canadian dry bean crop in 2002 (Manitoba Agriculture and Food, 2003). Bean production has increased substantially in the past five years. Since the only control of Aphanomyces is through crop rotation

and/or avoidance, root rot of bean should be closely monitored in order to avoid this species from developing to the same destructive levels as that seen in field pea.

In the course of this study, it was noticed that knowledge of *A. euteiches* root rot of field pea was quite limited. Producers were aware of the problem of root rot in pea but were unaware of the specific pathogens making up the pea root rot complex. Since available host resistance, fungicides and biological control agents have no effect on this pathogen, it will likely increase to destructive levels, as a direct result of this lack of knowledge. Therefore, it is crucial that more comprehensive studies on the distribution and host-range of this pathogen be undertaken in order to minimize losses through crop rotation and avoidance. Also, efforts should be made to increase farmers' awareness of this destructive pathogen in Manitoba.

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Appendix 1. Culture media and solutions

A.1.1. Water agar medium

Difco Agar	18 grams
Distilled water	1 L

A.1.2. Potato Dextrose Agar medium

Difco Potato Dextose Agar	39 grams
Distilled water	1 L

A.1.3. Maltose Peptone (3:1 percent solution) liquid media for zoospore production.

Difco Maltose	10 grams
Difco Bacto-Peptone	30 grams
Distilled water	1L

Dispense 50 ml in 250 ml flask

A.1.4. Sporulation wash solution for *Aphanomyces euteiches*.

CaCl2	1.75mM
MgSO4	1 mM
KCl	1 mM
Distilled water	1 L

Adjust pH to 6.5 with 0.01 M HCl or 0.01 N NaOH as required.

Reference: Mitchell and Yang, 1966. Pytopathology 56:917-922

Table A.2.1. Survey sites of Manitoba commercial pea field from 1999 and 2000. A list of the pathogens isolated from each site.

ID#	Location	Survey Date	Pathogens Isolated			
			A. euteiches	*Rhizoctonia*	*Fusarium*	*Pythium*
1	464 @ Hwy16, NE	7/14/1999				
2	464 @ 465, NE 6.5miles S Hwy16	7/14/1999				
3	250, 1.5miles S of of 24	7/14/1999				
4	24 @ 259, W of Rivers	7/14/1999				
5	259 @ 25, S	7/14/1999	Yes		Yes	
6	354, S of 24 1mile (Oak River)	7/14/1999		Yes		
7	264, S of 469 1mile, W side	7/14/1999		Yes		
8	264, S of 469 1mile, E side	7/14/1999		Yes		
9	264, N of 469 1 mile, W side	7/14/1999				
10	264, N of 469 2mile, N side	7/14/1999				Yes
11	Hwy83 E of 363, N side	7/14/1999				
12	Hwy83 S of Roblin .25mile, E side	7/14/1999				
13	Hwy83, S of Tummel, E side	7/14/1999			Yes	
14	Hwy83, S of Inglis, W side	7/14/1999			Yes	
15	Hwy83 @ Russell, W side	7/14/1999	Yes	Yes	Yes	
16	41, 2.5mile S Jct w/16, E side	7/14/1999			Yes	
17	41, 2.5mile S Jct w/16, W side	7/14/1999				
18	41, 3mile S Jct w/16, W side	7/14/1999			Yes	
19	41, 4mile S Jct w/16, W side	7/14/1999				
20	41, 4.5mile S Jct w/16	7/14/1999			Yes	
21	42 W shoal Lke, S side	7/14/1999			Yes	
22	432, 2mile S of 23, W side	7/14/1999	Yes		Yes	
23	432, 1mile S of 23, W side	7/14/1999	Yes		Yes	
24	Hwy3 E of Carman, N side	7/14/1999	Yes		Yes	Yes

ID#	Location	Survey Date	Pathogens Isolated			
			A. euteiches	*Rhizoctonia*	*Fusarium*	*Pythium*
25	Morden Research Station	Research plots 2000	Yes		Yes	
26	Penner's-N of Morden	Research plots 1999	Yes		Yes	Yes
27	Block 18, U of M-Point	Research plots 99/00	Yes		Yes	
28	Hwy41 S of Foxwarren	6/19/2000				
29	Hwy83 .5mile S of Roblin, E side	6/19/2000				
30	Togo-Richard's Dad's Farm	6/19/2000				
31	Hwy16 @ 41	6/19/2000				
32	41, .5mile S of Hwy16, E side	6/19/2000				
33	Markaroff Road, 1.5mile E of Togo	6/19/2000				
34	PR N of 42, toward Solsfirth 3 mile	6/19/2000				
35	Carberry, .5mile from rails, W side of 5	6/19/2000				
36	330, 1.5mile S of 205	6/19/2000			Yes	
37	205, 1.5mile W of Hwy75	6/19/2000			Yes	Yes
38	332, 2mile N of Starbuck	6/19/2000			Yes	Yes
39	13 @ Townlane 54N Road, N of Elmcreek	6/19/2000			Yes	
40	26, .5mile E of Poplarpoint	6/19/2000			Yes	Yes
41	248, 2.5mile N of Hwy1	6/19/2000	Yes		Yes	Yes
42	248, 1.5miles S of Ellie	6/19/2000			Yes	Yes
43	13, S of Hwy1, 1mile S of Oakville	6/19/2000				Yes
44	Hwy2, .5mile W of Springstien turnoff	6/19/2000				Yes

Table A.3.1. Duration required for sporulation of *Aphanomyces euteiches* isolates following the last rinse event with sporulation wash solution.

Isolate	Time (hours)
15	12
22	13
23	16
24	13
25	16
26	17
27	16
41	11
Average	~16

Table A.4.1. ANOVA table for disease severity. Pathogenicity experiment 1.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	200.97	28.71	44.41	<0.001
Degree	3	109.04	36.35	56.22	<0.001
Age	2	0.41	0.21	0.32	0.73

CV = 38.8

Table A.4.2. ANOVA table for root dry weight. Pathogenicity experiment 1.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	19.40	2.77	3.39	0.002
Degree	3	116.01	38.67	47.27	>0.001
Age	2	2.04	1.01	1.24	0.29

CV = 39.8

Table A.4.3. ANOVA table for shoot dry weight. Pathogenicity experiment 1.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	5.67	0.81	3.59	0.001
Degree	3	30.00	10.00	44.32	>0.001
Age	2	1.86	0.93	4.12	0.017

CV = 15.7

Table A.4.4. ANOVA table for disease severity. Pathogenicity experiment 2.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	124.49	17.78	19.92	>0.001
Degree	3	114.07	38.02	42.59	>0.001
Age	2	2.32	1.16	1.30	0.27

CV = 57.9

Table A.4.5. ANOVA table for root dry weight. Pathogenicity experiment 2.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	5.19	0.74	1.94	0.063
Degree	3	48.29	16.10	42.21	>0.001
Age	2	3.38	1.69	4.43	0.013

CV = 31.8

Table A.4.6. ANOVA table for shoot dry weight. Pathogenicity experiment 2.

Source of variation	df	SS	MS	F-statistic	P-value
Isolate	7	1.34	0.19	0.83	0.56
Degree	3	19.14	6.38	27.58	>0.001
Age	2	2.12	1.06	4.58	0.01

CV = 15.6

Table A.5.1. Effect of seedling age on disease severity (DS).

Experiment 1			Experiment 2		
Age (wks)	DS[1]		Age (wks)	DS	
2	2.1	a[2]	2	1.7	a
1	2.1	a	3	1.7	a
3	2.0	a	1	1.5	a
LSD	0.23		LSD	0.27	

[1] Average disease severity of 8 plants/pot, 3 replications.

[2] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table A.5.2. Effect of seedling age on root dry weight (RDW).

Experiment 1				Experiment 2			
Age (wks)	RDW[1](%)		RDW[2] (g)	Age (wks)	RDW[1](%)		RDW (g)
2	71.4	a[3]	2.35	2	89.6	a	2.07
3	76.2	a	2.31	3	106.0	ab	1.95
1	95.0	a	2.15	1	81.4	b	1.80
LSD			0.257	LSD			0.176

[1]Average root dry weight relative to the control. Determined by dividing the average root dry weight by the average control dry weight multiplied by 100.

[2] Average root dry weight of 8 plants/pot, 3 replications.

[3] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table A.5.3. Effect of seedling age on shoot dry weight (SDW).

Experiment 1				Experiment 2			
Age (wks)	SDW[1](%)		SDW[2] (g)	Age (wks)	SDW[1](%)		SDW (g)
3	60.0	a[3]	3.08	1	100.3	a	3.16
2	63.1	a	3.07	2	97.8	a	3.13
1	55.5	b	2.9	3	103.5	b	2.97
LSD			0.135	LSD			0.137

[1] Average shoot dry weight relative to the control. Determined by dividing the average shoot dry weight by the average control dry weight multiplied by 100.

[2] Average shoot dry weight of 8 plants/pot, 3 replications.

[3] Treatments with the same letter designation in the same column are not significantly different at p=0.05.

Table A.6.1. Average temperature for the 1999 growing season months at Winnipeg and Carman, Manitoba.

	Winnipeg (°C)	Carman (°C)
April	6.4	5.8
May	11.9	11.8
June	15.7	16.1
July	18.7	18.8
August	17.8	18.0
September	10.8	11.4
October	3.9	5.0
Total Average	12.0	12.4

Source: Environment Canada - National Climate Archive website: www.climate.weatheroffice.ec.gc.ca

Table A.6.2. Average precipitation for the 1999 growing season months at Winnipeg and Carman, Manitoba.

	Winnipeg mm	Carman mm
April	6.6	4.6
May	85.4	176.2
June	84.8	80.6
July	71.4	106.2
August	57.2	59.0
September	54.4	47.0
October	26.6	23.8
Total Average	55.2	71.1

Source: Environment Canada - National Climate Archive website: www.climate.weatheroffice.ec.gc.ca

Table A.6.3. Average temperature for the 2000 growing season months at Winnipeg and Morden. Manitoba.

	Winnipeg (°C)	Morden (°C)
April	3.6	5.2
May	10.6	12.4
June	14.2	15.5
July	19.1	19.9
August	18.5	19.9
September	11.3	12.9
October	6.1	7.7
Total Average	11.9	13.4

Source: Environment Canada - National Climate Archive website: www.climate.weatheroffice.ec.gc.ca

Table A.6.4. Average precipitation for the 2000 growing season months at Winnipeg and Morden, Manitoba.

	Winnipeg mm	Morden mm
April	4.6	4.0
May	48.4	47.2
June	175.8	90.8
July	102.4	39.0
August	67.0	166.4
September	63.2	40.2
October	30.4	19.6
Total Average	70.3	58.2

Source: Environment Canada - National Climate Archive website: www.climate.weatheroffice.ec.gc.ca