Occurrence and management of root rot of field pea caused by *Aphanomyces euteiches*

by

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Abstract

Aphanomyces euteiches causes Aphanomyces root rot (ARR), an important disease of field pea (*Pisum sativum*). The development of ARR results in severe root damage, wilting and large yield losses under wet soil conditions. A survey of field pea crops in central Alberta in 2016 found an average incidence of root rot of 57.6%, ranging from 2% to 100%. Species of *Fusarium* were recovered most frequently from symptomatic roots, followed by *Pythium* spp., *A. euteiches* and *Rhizoctonia* spp. Inoculum density experiments under greenhouse and field conditions demonstrated an adverse effect of increasing *A. euteiches* inoculum concentration on pea seedling emergence, root nodulation and plant vigor. Seed treatment with the fungicides Apron Advance (thiabendazole + fludioxonil + metalaxyl) + Vibrance (difenoconazole+ metalaxyl-M+ sedaxane), Intego Solo (ethaboxam), BAS 516F (boscalid + pyraclostrobin), BAS 720F (metalaxyl + pyraclostrobin + fluxapyroxad) or BAS 516F + BAS 720F (3:1) were evaluated for their efficacy against ARR. All seed treatments except Apron Advance suppressed ARR development under controlled conditions. Twenty-two pea lines and cultivars were evaluated for resistance to ARR in field plot experiments, with the genotype 00-2067 found to be most tolerant to the disease. A recombinant inbred line (RIL) pea population was obtained by single-seed descent from the cross 00-2067 (ARR tolerant) × Reward (ARR susceptible) and used to identify quantitative trait loci (QTLs) associated with root rot severity, height, vigor, root weight and foliar weight in greenhouse and field trials. A total of 212 simple sequence repeat (SSR) markers were screened by bulk segregant analysis and the polymorphic markers

used for linkage map construction. Composite interval mapping identified a total of six QTLs associated with tolerance to ARR, root weight, foliar weight and height. Two of the QTLs, *PARR-LGI* and *PRW-LGI* on linkage group I (LGI), explained 52.5% and 34.2% of the variation in root rot severity and root weight, respectively, in one greenhouse replication, while the four other QTLs, *PRW-LGII*, *PFW-LGII-1*, *PFWLGII-2* and *PH-LGII* on linkage group II (LGII), explained 14.0-17.1% of the phenotypic data in greenhouse and field experiments. The results of this study suggest that an integrated approach, which incorporates the use of seed treatments and QTLs associated with tolerance to the ARR, will be required for the sustainable management of ARR of field pea in Canada.

Preface

This thesis is an original work by Mr. Longfei Wu. I carried out the research and completed the first drafts of the thesis chapters. Each chapter was then reviewed and edited by Dr. Stephen E. Strelkov, Dr. Sheau-Fang Hwang and Dr. Kan-Fa Chang, who also developed the original research concept. Dr. David Feindel (Alberta Agriculture and Forestry) participated in the design of the greenhouse and field experiments and provided suggestions during the course of program. Dr. Robert Conner (Agriculture and Agri-Food Canada, Morden, MB), a collaborator, also reviewed Chapters 3 and 4 and provided suggestions on data presentation. Dr. Rudolph Fredua-Agyeman (Alberta Agriculture and Forestry) provided assistance with the data analysis and editorial suggestions in Chapters 3 and 4. My supervisor Dr. Strelkov, in collaboration with Dr. Hwang and Dr. Chang, provided me with guidance and corrections throughout the thesis.

While I was responsible for the data collection and analysis, I received assistance from research personnel and summer students (University of Alberta, Alberta Agriculture and Forestry) in establishing and maintaining the field and greenhouse experiments, as well as in the root sample collection.

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List of Abbreviation

AFLP	amplified fragment length polymorphism
AM	Arbuscular mycorrhizal
ANOVA	analysis of variance
ARR	Aphanomyces root rot
CFU	colony-forming unit
CIM	composite interval mapping
cM	centimorgans
DS	disease severity
DSI	disease severity index
GGE	genotype-by-environment
GWAS	genome-wide association study
ISSR	inter simple sequence repeat
LG	linkage group
LOD	Logarithm of odds
LSD	least significant differences
MAS	marker-assisted selection
MBV	metalaxyl-benomyl-vancomycin
NIL	near-isogenic line
PCA	principal components analysis

PDA potato dextrose agar

PI plant introduction

QTL quantitative trait loci

RAPD random amplification of polymorphic DNA

RCBD randomized complete block design

RIL recombinant inbred line

SNP single-nucleotide polymorphism

SSR Simple sequence repeat

STS sequence tagged site

Chapter 1. General introduction and literature review

1.1. Pea

Field pea (*Pisum sativum* var. *arvense* L.), along with common bean (*Phaseolus vulgaris* L.), field bean (*Vicia faba* L.), soybean (*Glycine max* (L.) Merr.), chick pea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.), belongs to the Leguminosae family. Pea seeds contain 20-30% total protein, are rich in carbohydrates, calcium, iron, phosphorus, and various vitamins, and provide a protein-rich food and feed source for humans and animals. Field pea is one of the most widely cultivated crops on the Canadian prairies, with exports of 2.6 million metric tons representing $870 million in revenues for Canadian farmers (Agriculture and Agri-Food 2017).

1.1.1. Biology of pea

Although pea is one of the most well-characterized legume species, and has been used as a model in many genetic, biochemical and physiological studies, it is still difficult to provide a general description of this plant due to the large variation in morphology and physiology within the species *P. sativum* (Hagedorn, 1984). Pea may be simply described as a cool-season, herbaceous, annual legume with a bushy climbing nature. To facilitate management in pea production, Knott (1987) classified pea development into four growing stages: germination and emergence, vegetative, reproductive and senescence.

During the germination stage, the cotyledons provide nutrients to support the growth of the gemmule. The root system then develops and spreads in the soil, followed by secondary root branching from the taproot. When infected with *Rhizobium* bacteria, pea roots may form

nodules, which are able to fix nitrogen directly from the atmosphere. The leaves of peas are pinnately compound, with two large leaf-like stipules at the base. The terminal tendrils are arranged at the end of the petiole. At the reproductive stage, inflorescences form in the leaf axis. The self-pollinating flowers have five bilaterally symmetrical petals that may be white, pink or purple. At the senescent stage, physiological maturity of the pod occurs 24-30 days after self-pollination. Normally, 5-6 seeds are produced in each pod, but the number of seeds varies depending on the cultivar and growing conditions. Dry seeds may be green, white, olive, brown orange or reddish-brown in color, and may sometimes have a speckled or marbled appearance. The edible seed consists of two fleshy cotyledons and an embryonic axis. Programmed cell death in the funiculus, which provides the connection between the pods and leaves, occurs when the pods are air-dried naturally. Most cultivars that are consumed fresh as a vegetable or in food processing (i.e., canning and freezing) have wrinkled seeds and can be harvested when the green, enlarged ovules are sweet. Cultivars used for dry seed purposes are usually harvested at maturity.

1.1.2. History of pea cultivation

Peas made their first appearance in early Neolithic farming villages in the Near East dating from 7000 to 6000 B.C. More frequent pea cultivation occurred during the Neolithic period in the European agricultural settlements in Greece (5500 B.C.), Bulgaria (4330 B.C.), Romania, Czechoslovakia, Austria, Switzerland, and the Rhine Valley (4400 - 4200 B.C.) (Zohary and Hopf 1973). There are two types of wild peas, which are genetically related to the modern pea: a

tall omni-Mediterranean wild pea (*P. elatius* Beib.) with large purple-blue flowers and a smaller wild pea (*P. humile* Boiss. et Noe). Perhaps the most reliable indication of the pea domestication process was the change of the seed coat, from a rough or granular seed surface to a smooth surface (Zohary and Hopf 1973). According to Abbo et al. (2011), the wild pea suffered yield loss due to poor establishment caused by low germination rates and seed hardness. The origin and evolution of pea from the wild-type to the modern type provide possibilities for the study of the genetic composition and adaption of pea to different environments.

1.1.3. History of pea production

World grain pea production was around 11 million tons in 2014 (Table 1.1). This represents a decrease from 1990, when the highest worldwide production (16.6 million tons) was recorded, mainly due to a reduction in pea cultivation in Europe (FAOSTAT 2017). In contrast, there has been a significant increase in pea cultivation in North America (Canada and USA) starting in the1990s.

Pea was introduced to Canada over 100 years ago (Slinkard et al. 1994) and first grown in some limited areas in eastern Canada in the late 1800s. In 1985, there were only 80,500 ha of field peas seeded in Canada. Due to its adaptation to cool climates and its high nutritional value for human and livestock consumption, field pea has become increasingly popular as a cash crop in Canadian cropping systems. In 1987, 300,000 ha were seeded to field pea in Canada (Ali-Khan and Zimmer 1989). By 2014, Canada was the largest producer of field peas (3,444,800 ha) and accounted for 21% of total world production (FAOSTAT 2017).

Western Canada became the main field pea growing region in the 1980s. Due to the increasing world demand for field pea and low returns for cereal grains, large increases in field pea production were recorded in all three Prairie Provinces over the past few decades. Alberta farmers first grew field pea with conventional farm machinery in 1980 with a total seeded area of only 43 ha (Anonymous 1996). In 1987 and 1988, 8,000-12,000 ha of green-seeded field peas were grown in Alberta. By 2011, Alberta had become the second largest pea cultivation region in Canada (348,965 ha) after Saskatchewan (1,711,497 ha) (Bekkering 2011). The yields of field peas have increased continuously due to the application of new management practices and planting of high yielding cultivars with the feature that include an erect and semi-leafless stand. Under favourable soil and weather conditions, yields of peas may reach 2500 kg/ha in the major pea-growing areas (Ali-Khan and Zimmer 1989). Pea production is well-established in Alberta, with the highest pea yields in Canada, averaging 35% higher than either Saskatchewan or Manitoba (Paul 1986). From 1999 to 2008, Alberta produced 21% of the field pea in Canada (Wang 2009)

Pea production has brought significant benefits to Canadian farmers. More than 2.6 million metric tons of Canadian field peas are exported to Europe, South America and Asia on an annual basis (Agriculture and Agri-Food Canada 2017). In addition, peas also are used for domestic consumption. Green peas usually are used for making soups. In Alberta and Manitoba, there is also a large demand for field peas from the pork industry, in which the peas are utilized as feed (Anonymous 1996). In recent years, the processing industry in Manitoba has used pea to

produce food products with various protein, starch, and fibre fractions from pea flour for food enrichment and industrial applications.

1.2. Aphanomyces root rot

1.2.1. Root rot complex in pea

The pea root rot complex (PRRC) has been reported to be a serious problem in field pea production worldwide, including in Canada (Tu and Findlay 1986; Pfender et al. 2001). Usually, the PRRC involves several soil-borne pathogens including *Aphanomyces euteiches*, *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., and *Rhizoctonia solani* (Bailey et al. 2003; Chang et al. 2005, 2013). Hwang and Chang (1989) reported that this disease complex was prevalent in Alberta as early as the 1980s. A loss of 24,000 tons of field pea was caused by PRRC in southern Ontario in 1983 (Tu 1987). *Fusarium solani* is the most prevalent causal agent of pea root rot worldwide (Ondrej et al. 2008). However, *F. avenaceum* was reported to be the principal pathogen causing Fusarium root rot of pea in Alberta, accounting for 80% of the isolates collected from field samples (Feng et al. 2010). According to Tu (1994), the prevalence of *Fusarium* spp. could be influenced by soil compaction, temperature, and moisture. The occurrence of *A. euteiches* has been reported in certain North American and European countries, as well as Japan, Australia and New Zealand (Wicker et al. 2003). *Aphanomyces euteiches* was considered to be one of the causal agents of pea root rot in Canada as early as the 1970s (Basu et al. 1973). It was not, however, suggested to be a prevalent or severe pathogen in this country until recently (Gossen et al. 2016). It was first confirmed on lentils in Manitoba in 2012

(Armstrong-Cho et al. 2014) and on field pea in Alberta in 2013 (Chatterton et al. 2015). When PRRC is severe, reductions in yield can be as high as 70% (Tu 1987; Hwang et al. 1991).

PRRC causes several symptoms including seed decay, damping-off, seedling blight, root rot, and wilt, but it is difficult to differentiate the causal organisms solely by examining the symptoms (Xue 2003a). This increases the difficulty associated with predicting and managing PRRC in Canada. Seed decay results in the soft, mushy appearance of seeds and their rapid deterioration. Direct invasion of the seeds by any of the fungi, but most often by *Pythium* spp., is usually the cause of seed decay (Schrum et al. 2008). Damping-off and seedling blight reduce seedling emergence and plant density, limit pea growth, delay canopy closure and therefore increase weed competition. All of these may cause yield reductions (Lawson and Topham 1985). Root rot also restricts the transport of water and nutrients in pea roots, and reduces canopy density and the uniformity of crop maturity (Xi et al. 1995). Root rot can also cause reddish brown discolorations, which spread from the stem just below the soil to the roots (Chang et al. 2005). Root rot also may destroy *Rhizobium* nodules, leading to a reduction in nitrogen fixation in the root (Hwang et al. 2003).

1.2.2. Oomycetes: plant and animal disease-causing agents

Oomycetes are a large group of eukaryotes (ca. 600 to 1,500 species) including the most diverse, important, and the earliest known water molds, which usually refers to members of the Saprolegniales, which cause severe damage to various crops. Oomycetes resemble fungi in morphology (i.e., mycelial growth) and many have a parasitic lifestyle. They are heterotrophic

organisms absorbing nutrients from host cells by releasing depolymerizing enzymes that degrade extracellular complex biological materials of the host. Oomycetes usually form coenocytic and complex branched mycelia that grow from the hyphal tip. Because of these characteristics, oomycetes were considered as members of the Kingdom Fungi for many years. Copeland (1956), Gaumann (1952, 1964) and Sparrow (1976) were among the first to distinguish oomycetes from other fungi. Shaffer (1975) excluded oomycetes from the Kingdom Fungi. It is now clear that they are phylogenetically different from the fungi and are more closely related to diatoms, chromophyte algae and other heterokont protists.

The most ancestral oomycetes known today (i.e., *Eurychasma dicksonii* and *Haptoglossa* spp.), which are most similar to brown algae, have aquatic habitats with both saprotrophic and parasitic lifestyles (Beakes et al. 2012). Unlike true fungi, oomycetes produce motile, biflagellate zoospores (Alexopoulos and Mims 1979). Cytological and biochemical studies indicate more differences that distinguish oomycetes from fungi. In the vegetative stage, the mycelium of oomycetes consists of a coenocytic thallus that remains diploid. The formation of haploid nuclei only occurs through meiosis for gamete formation. At this stage, fungal thalli become septate just below the reproductive organs and each cell unit carries one haploid nucleus. In addition, while fungal cell walls are composed mainly of chitin (acetylglucosamine polymers), glucans, polysaccharides and mucopolysaccharides, waxes, and pigments, cell walls of the oomycetes present a more diverse polymer composition. In late-divergent oomycetes, such as *Phytophthora infestans*, cellulose and β-glucans are the principal structural cell-wall

components, whereas early-divergent species like *Saprolegnia* spp. and *A. euteiches* also contain cellulose, as well as various portions of chitin (Badreddine et al. 2008; Guerriero et al. 2010). Recent studies also detected unique polymers: 1,6-linked GlcNac residues in association with β-1,6 glucans, a unique composition first reported in eukaryotes (Mélida et al. 2013; Nars et al. 2013). In addition, oomycetes cannot produce sterols and need to acquire them from hosts through the secretion of sterol-carrier proteins during infection, which indicates the absence of the corresponding biosynthetic pathways in this group. Molecular phylogenetic studies based on the mitochondrial cox2 gene (Thines et al. 2008), LSU rDNA genes and ITS sequences (Leclerc et al. 2000) place the oomycetes in the super ensemble Chromalveolata, far distant from true fungi.

A key feature of oomycetes is their reproductive process. In the sexual stage, the oomycetes produce a female oogonium, which is fertilized by the male antheridium to form unicellular oospores. Oospores can endure unfavorable conditions and survive for long periods of time. The asexual spores, or zoospores, consist of a wall-less cell equipped with two flagella for movement in the water. Oomycetes also form an asexual structure, called a sporangium, to form and release zoospores. In some air-borne species, the sporangia are released easily from the mycelium and spread by the wind. However, the sporangia of some species in the genus *Peronospora* (causing downy mildew diseases) have lost the ability to produce and release zoospores (Cooke et al. 2000). Oomycetes may be homothallic or heterothallic. There is also an asexual survival

structure, called a chlamydospore, produced by some oomycetes, which is an asexual resting spore with a thickened cell wall.

Sparrow (1976) divided the oomycetes into six groups: Eurychasmales, Saprolegniales, Lagenidiales, Peronosporales, Thraustochytriales and Labyrinthulales. There are two major taxonomic groups/lineages out of the six groups described by Sparrow (1976): the Saprolegniales and the Peronosporales (Fig. 1.1). The Saprolegniales predominantly occur in aquatic environments (fresh water and estuarine) and can be isolated easily, while the Peronosporales occupy mainly aquatic, amphibious and terrestrial environments. Saprolegniales usually are characterized by a profusely branched, coenocytic mycelium. Their hyphae usually contain no septa except the position just below the reproductive organs. Peronosporales consist of a well-developed mycelium, from which the coenocytic, stout hyphae branch freely (Alexopoulos and Mims 1979). Phytopathogenic species of oomycetes are present in most Peronosporalean lineages and some of the Saprolegnialean lineages (Fig. 1.1). Hyphae of these pathogens are intercellular or intracellular. Phytopathogenic species in the Peronosporalean lineage include the genera *Phytophthora* (over 100 species, Kroon et al., 2012), *Albugo*, *Hyaloperonospora*, and *Pythium*. Some animal pathogens also are included in the genus *Pythium*, for example, *P. insidiosum* (Uzuhashi et al. 2010). In the Saprolegnialean lineage, plant pathogens have only been identified in the genus *Aphanomyces*.

1.2.3. The genus *Aphanomyces*

Aphanomyces is a diploid, homothallic genus of organisms producing oospores and zoospores which is related most closely to the brown algae within Stramenopiles (Leipe et al. 1994). Compared with other genera, such as *Saprolegnia*, *Dictyuchus*, and *Achlya*, *Aphanomyces* constitutes an ancestral group (Cooke et al. 2000; Leclerc et al. 2000), as indicated by the characteristic of an achlyoid spore dehiscence type (Scott 1961). The genus *Aphanomyces* includes a number of water mold species existing as saprophytes or parasites of fish, crayfish and plants (Grünwald 2003). There are around 40 described species of *Aphanomyces* (Diéguez-Uribeondo et al. 2009), which are further grouped into three independent lineages based on their life style (Fig. 1.1). Most have a wide range of hosts belonging to different families (with a few exceptions such as *A. cochlioides* on sugar beet (*Beta vulgaris* L.) (Diéguez-Uribeondo et al. 2009) and *A. iridis* on iris (Grünwald 2003)). A second lineage harbours species with a prevalence for saprophytism such as *A. laevis* and *A. helicoides*, which can exhibit opportunistic parasitism. Lastly, the zoopathogenic lineage usually occurs in aquatic niches (fresh water and marine, mostly estuarine), such as *A. astaci* infecting freshwater crayfishes (Filipová et al. 2013), *A. invadans* infecting various species of estuarine fishes (Boys et al. 2012), and *A. stellatus*, which has been reported to develop on crustaceans as a saprotroph (Royo et al. 2004).

1.3. Aphanomyces root rot caused by *A. euteiches*

1.3.1. The incidence of Aphanomyces root rot

Since it was first described by Jones and Drechsler (1925) and extensively reviewed by Papavizas and Ayers (1974), *A. euteiches* has been considered as one of the most damaging soil-borne pathogens on legumes. At present, *A. euteiches* infections have been reported in all of the main pea cultivation regions of the world (Wicker et al. 2003). In France, it affects primary forage pea in the northern regions (Gaulin et al. 2007). In North America, it causes severe disease in the Great Lakes region, the northeastern United States (Pfender 1984) and the Pacific Northwest (Kraft 1992). Yield losses caused by *A. euteiches* can be as high as 86% in some heavily infested pea fields (Pfender and Hagedorn 1983).

1.3.2. Favourable conditions for disease development and symptomatology

Aphanomyces root rot can develop within 7-14 days after first infection, depending on soil moisture, temperature and the concentration of oospores (Papavizas and Ayers 1974; Pfender and Hagedorn 1983). Undoubtedly, high *A. euteiches* inoculum densities increase the incidence and severity of ARR. Chan and Close (1987) observed a positive correlation between the number of oospores per 100 g soil and root rot severity. Splashing rain is important for infection by *A. euteiches*, as well as for its development and dissemination. The minimum soil moisture level required for initiation of root rot disease is around 30% water holding capacity (Haenseler 1926; Smith and Walker 1941). Oospores can germinate to form germ tubes which directly penetrate the roots. Soil moisture also stimulates the germination of oospores to form sporangia, which release zoospores that travel in the moisture films surrounding soil particles to the plant

roots (Scharen 1960; Hoch and Mitchell 1973). Soil moisture also facilitates the leakage of metabolites from pea roots (Kerr 1964), which stimulate the germination of oospores and attract zoospores (Pfender et al. 2001). Although high rainfall favours ARR outbreaks, completion of the infection process by *A. euteiches* only requires a short period (Pfender 1984). Aphanomyces root rot may occur over a wide temperature range, which also is conducive to pea growth (Pfender 1984), but the optimal temperature is about 16°C for infection and 20-28°C for disease development (Burke and Mitchell 1968; Burke et al. 1969). Hot temperatures may accelerate pea root decay when infected by *A. euteiches*, which further limits water and nutrient transport in pea plants.

Gaulin et al. (2008) reported that *A. euteiches* could infect legume hosts at any growth stage, but others pointed out that infection occurs more commonly at the seedling stage (King and Parke 1993; Kraft and Kaiser 1993). Infection is initiated on the root surface by zoospores, which are chemo-attracted by root exudates (Sekizaki et al. 1993), encyst in the rhizoplane and germinate to penetrate root cortex tissues. The pathogen can colonize entire root systems, and can spread from the roots to the stems (hypocotyls, epicotyls) . Infected seeding roots become soft and water-soaked with a honey-brown or blackish-brown color, which turn orange-brown or blackish-brown at later stages of disease development. The final stage of infection is marked by the formation of oospores which serve as inoculum for future infections. Water transport within affected plants is reduced significantly when symptoms are severe (Muehlchen et al. 1990). This can result in above-ground symptoms that include dwarfing, wilting, and premature death

(Chupp and Sherf 1960). Chupp and Sherf (1960) also indicated that ARR can cause severe delays in pea maturity, reducing the number of seeds per pods and resulting in poor seed quality. Sherwood and Hagedorn (1962) developed a five-point scale (0-4) for rating ARR severity, which can be used to calculate a disease severity index (DSI); this rating system has been used widely in later studies.

1.3.3. Host range

Aphanomyces euteiches has a broad host range in the legume family (Papavizas and Ayers 1974; Moussart et al. 2007). It has been isolated from pea, alfalfa (*Medicago sativa*), snap and red kidney beans (*Phaseolus vulgaris*), faba bean (*Vicia faba*), red clover (*Trifolium pratense*), white clover (*T. repens*), and several other weed species (Gaulin et al. 2007). Nevertheless, its occurrence and degree of pathogenicity may differ from one host to another. Pea-infecting strains and alfalfa-infecting strains from the US and from France have been identified (Malvick and Grau 2001; Wicker et al. 2001; Moussart et al. 2007). Papavizas and Ayers (1974) also noted large economic losses in North America and Europe due to infection of pea and alfalfa by *A. euteiches*. The wide host range of *A. euteiches* makes the management of ARR by crop rotation difficult.

1.3.4. Disease cycle

Aphanomyces euteiches has both asexual and sexual stages in its life cycle in the soil, which allows efficient dissemination (zoospores) and survival from harsh winter conditions (oospores)

(Gaulin et al. 2007). The oospores are 20-35 µm in diameter, have a thick protective wall and contain energy reserves in the form of a large oil globule. The oospores, which can survive in the soil for over 10 years (Pfender and Hagedorn 1983), may spread over long distances by the transportation of infested soil and/or infected plants (Papavizas and Ayers 1974). When attached to the pea roots, oospores may germinate under conductive temperature and moisture conditions and form either a mycelium or zoosporangium. The zoosporangium, which is formed through a long tube on the oospores, may release large numbers of zoospores (Scott 1961). Subsequently, the biflagellate motile zoospores, which are attracted by chemical signals in plant root exudates, find a suitable host (Sekizaki et al. 1993). The motile zoospores locate and encyst on the host roots within minutes, with the resulting cysts germinating and penetrating the host cortical cells within hours (Papavizas and Ayers 1974). After the initial infection and penetration, coenocytic hyphae develop rapidly in the intercellular spaces within the host. A few days after infection, the sexual stage of *A. euteiches* may be produced, with the formation and fusion of antheridia and oogonia (Scott 1961). The thick-walled oospores are formed as a result of sexual reproduction and are released into the soil as the roots decompose, serving as the primary source of inoculum for new infections (Mitchell and Yang 1966) (Fig. 1.2).

1.3.5. Variability and physiologic specialization

Information on pathogenic variability and physiologic specialization in *A. euteiches* is limited due to a lack of completely resistant or immune pea genotypes. However, differences among isolates have been identified based on zoospore size, time required for sporulation and ability to

produce zoospores, growth rate on culture media, oospore size and the amount of pectinolytic and cellulolytic enzymes produced (Papavizas and Ayers 1974). Physiologic specialization was first examined by King and Bissonette (1954), who indicated that isolates of *A. euteiches* differed in their virulence patterns on various pea cultivars in Minnesota. Carlson (1965) tested 10 isolates of *A. euteiches*, which were isolated from infested soil collected in Minnesota, New York and Wisconsin, by inoculating the root tips of tolerant and susceptible pea cultivars, and reported considerable differences in the ability of isolates to infect plants and produce oospores. Differences in virulence and growth characteristics also were observed among seven single-zoospore isolates obtained from germinated oospores (Scharen 1960). Beute and Lockwood (1967) inoculated six differential cultivars with 15 *A. euteiches* single-zoospore isolates, and identified two races based on their virulence on these pea cultivars. Sundheim and Wiggen (1972) first confirmed the existence of physiologic races of *A. euteiches* in Europe with the same set of differential pea cultivars described by Beute and Lockwood (1967). Sundheim and Wiggen (1972) also identified multiple races of the pathogen from 14 isolates, although the criteria used to distinguish these races was questioned by Manning and Menzies (1984). The inconsistencies between studies highlight the difficulties involved in characterizing the race structure of *A. euteiches.*

More recently, Malvick and Percich (1998) used a new differential set (consisting of the pea genotypes MN313, MN314, 90-2079, WI-8904, Little Marvel, Saranac and Early Gallatin) to evaluate pathogenic variability in 114 *A. euteiches* strains. These researchers determined that

all strains were pathogenic on one or more pea hosts, while 18% and 14% were pathogenic on alfalfa and bean, respectively. They concluded that *A. euteiches* populations were genotypically and phenotypically variable in the central and western United States. Four virulence groups were identified in a subsequent study, which used a disease severity (DS) > 3.0 on a 0-4 scale to indicate a clear pathogenic interaction (Malvick and Percich 1999). Later, Wicker and Rouxel (2001) examined 109 isolates collected from France, Denmark, Sweden, Norway, USA, Canada, and New Zealand using another differential set (consisting of the peas Baccara, Capella, 902131, MN313, 552 and PI180693), and identified 11 virulence types; in that study, the pea genotype 902131 consistently showed resistance to three Canadian isolates. Wicker and Rouxel (2001) also used disease severity index (DSI) based on a 0-5 scale and considered incompatibility (DSI <1) as a resistance response, with all other ratings taken as a compatible reaction. To accurately evaluate the virulence of *A. euteiches* strains from different countries, Wicker et al. (2003) evaluated 33 pea lines on the five selected differentials that were described by Wicker and Rouxel (2001). Studies of resistance in differential pea genotypes have contributed to the development of commercial pea cultivars with ARR resistance (Wicker et al. 2003). Nonetheless, further studies examining more isolates of *A. euteiches* from a broader geographical area are required to better understand physiologic specialization in this pathogen.

1.4. Disease management

Aphanomyces root rot has been recognized as one of the most damaging root diseases of field pea over the past 100 years. The management of this disease, however, still needs to be

improved. No pea cultivars completely resistant to ARR are available (Pfender 1984; Allmaras et al. 2003) and only partial resistance and/or tolerance have been reported in several studies (Conner et al. 2013; Hamon et al. 2013; Lavaud et al. 2015). Fungicidal seed treatment only improves plant health at the seedling stage, but efficient fungicide applications for protection after the seedling stage are not commercially available. At present, the strategy recommended most commonly used to manage ARR is disease avoidance via methods such as crop rotation (Vandemark et al. 2000).

1.4.1. Cultural practices

Crop rotation is one of the most fundamental and oldest methods to manage diseases caused by soil-borne pathogens, but its effectiveness directly coincides with the length of rotation (Garrett 1944). Jones and Linford (1925) demonstrated a positive relationship between the number of pea crops and root rot severity, and hypothesized that rotation with non-host crops may help reduce the density of *A. euteiches* in the soil and thereby reduce the severity of ARR. The first systematic study of the effectiveness of crop rotation patterns on ARR was conducted by Temp and Hagedorn (1967), who found that long-term crop rotations could reduce *A. euteiches* inoculum density in the soil, but were not always effective in eradicating the disease. Olofsson (1967) questioned the practicality and effectiveness of crop rotation as a method to manage ARR, because the oospores can survive 10-15 years in the absence of a host. Furthermore, many alternative host species can sustain inoculum levels in the absence of pea. According to Hossain et al. (2014), however, hydrolysis products of Brassica cover crops significantly suppressed the

pathogen, and they recommended a crop rotation interval of 6-8 years. Williams-Woodward et al. (1997) examined the effect of oats (*Avena sativa* L.) as a rotation crop with pea, and found that oat residues improved soil suppression of ARR. Therefore, increased crop diversity may represent a good long-term strategy for disease management (Krupinsky et al. 2002).

Soil conditions can be suppressive or conducive to ARR, and can play an important role in the management of pea root rot caused by *A. euteiches* (Oyarzun et al. 1997). Heyman et al. (2007) examined the relationship between soil nutrients (Ca, Mg, K and P) and pH with ARR severity and observed a strong negative correlation between Ca concentration and disease development, suggesting that free Ca was a major variable in controlling the degree of soil suppressiveness to *A. euteiches*. They further suggested that Ca might play a role in the inhibition of zoospore production from the oospores (Heyman et al. 2007).

Residues from two plant families have been reported to reduce the severity of ARR: the Brassicaceae (crucifer family) and the Poaceae (grass family). In the Brassicaceae, the residues of cabbage (*Brassica oleracea* var. *capitata* L.), kale (*B. oleracea* L. *acephala* DC.), mustard (*B. nigra* L.), white mustard (*Sinapis alba* L.), turnip (*B. rapa* subsp. *rapa* L.), and rape (*B. napus* L.) have been reported to suppress the incidence of ARR on field pea (Papavizas1966, 1967; Papavizas and Lewis 1971; Chan and Close 1987; Muehlchen et al.1990). Similarly, oats (*Avena sativa* L.), rye (*Secale cereale* L.), corn (*Zea mays* L.), and Sudan grass (*Sorghum halepense* L. Pers.), and other members of the Poaceae have been used as green manure crops to control this

disease (Davey and Papavizas 1961; Tu and Findlay 1986; Tu 1990, 1992; Fritz et al. 1995; Williams-Woodward et al. 1997).

According to Fritz et al. (1995), soil compaction can enhance the development of ARR, resulting in yield losses as high as 63%. In contrast, the yield of plots covered with oat shoots and residues increased by 48% in the same disease nursery, suggesting a promising method for the cultural control of ARR on pea. Allmaras et al. (2003) also confirmed the effect of oats as a pre-crop in suppressing ARR and pointed out that excessive compaction related to tillage and traffic management may impair internal drainage and thus adversely reduce the effect of oat residue in controlling this disease.

Field indexing by sampling soils to determine the inoculum potential of *A. euteiches* also can be an effective method to manage ARR of field pea prior to seeding. Studies have identified and distinguished severely infested fields from non-infested or mildly infested fields (Sherwood and Hagedorn 1958; Reiling et al. 1960). Such information can be used to make appropriate crop management decisions, such as the exclusion of pea from fields known to be severely infested with ARR.

1.4.2. Molecular detection of *A. euteiches*

Molecular markers are useful tools for the identification of fungal pathogens. Testing of soil or plant samples for the presence of *A. euteiches* DNA by PCR analysis with species-specific primers has been used widely in the identification of *A. euteiches* (Vandemark et al. 2002). Chatterton et al. (2015) detected *A. euteiches* in pea fields in Alberta based on a PCR assay. A

number of commercial kits also have been developed to identify *A. euteiches* efficiently. However, information on the use of molecular markers for the identification of races or pathotypes of *A. euteiches* is still limited and preliminary. Malvick and Percich (1998b) conducted random amplified polymorphic DNA (RAPD) analysis to evaluate genotypic diversity among strains of *A. euteiches* in the USA, but found that only four of 76 polymorphic RAPD markers tested were associated with pathogenic variation. In a separate study, the same researchers successfully distinguished one major group and two closely related minor groups of strains among 114 isolates of *A. euteiches* collected from four locations in the USA (Malvick and Percich 1998a). Sauvage et al. (2007) identified two sets of primers, 136F/136R and 11F/280R, that were able to amplify different sized PCR products from a collection of 105 *A. euteiches* isolates. Unfortunately, the relationship between the fragments amplified by the two sets of primers and the growth and virulence features of the corresponding *A. euteiches* isolates was not analyzed in that study.

1.4.3. Seed and soil treatments

While seed treatments are used commonly in western Canada to improve pea seedling emergence, there have been no reports on fungicides that can effectively control ARR (Papavizas and Ayers 1974). Tu (1992) pointed out the limitations to the control of pea root rot with capton (ethanethiol or ethyl mercaptan), which was introduced as a foliar fungicide for fruit crops and as a seed treatment for corn (Daines 1953). Differences in cell wall composition and

metabolic pathways between oomycetes and the true fungi make *A. euteiches* insensitive to most fungicides. According to Bruin and Edginton (1983), neither the systemic acylalinine-type of oomycete fungicides, such as metalaxyl, nor the ethyl phosphonates, such as fosetyl-AI, or cymoxanil, can control ARR effectively. Some chemicals that suppress *A. euteiches* effectively under controlled conditions have limited beneficial effects in field trials (Oyarzun et al. 1990; Xue 2003b). Tachigaren (hydroxyisoxazole or hymexazol) is reported to reduce root rot severity and increase yield under experimental field conditions (Kotova and Tsvetkova 1980) and is available in Japan for the control of *Pythium* and *Aphanomyces*-diseases of sugar beets. However, the effectiveness of Tachigaren for the control of ARR was variable in other studies (Jermyn et al. 1982; Gritton et al. 1995). Up to now, only INTEGO Solo (ethaboxam) is registered for *Pythium* control and suppression of seed rot caused by *Phytophthora* and *Aphanomyces* spp. in legumes in Canada.

1.4.4. Biological control

Biological control offers a promising strategy for the management of ARR. Hence, it has received significant attention in recent years. Antagonistic microorganisms, which are applied to the pea seeds or the soil, may help protect plants from infection by fungal or fungal-like pathogens. The spores of arbuscular mycorrhizal fungi and some spore-forming bacteria can suppress mycelial growth and germination of spores of *A. euteiches* (Wakelin et al. 2002; Karin et al. 2004). Application of isothiocyanate, a compound produced in shoots of the Brassicaceae,

also has been shown to have potential in the control of ARR of pea, due to its toxic effect on *A. euteiches* (Hossain et al. 2014).

Biocontrol agents can be used in conjunction with fungicidal seed treatments. Recent studies have demonstrated that strains of some bacteria, including *Gliocladium roseum*, *Pseudomonas fluorescens* and species in the *Burkholderia cepacian* complex, improve seedling emergence in fields infested with *A. euteiches* when formulated as a seed coat application together with a fungicide (Parke et al. 1991; Bowers and Parke 1993; Xue 2003b). Xue (2003b) studied seed treatments consisting of the biocontrol agent *Clonostachys rosea* and the fungicides Thiram 75WP or Apron FL and found that they contributed to improve seed germination of pea in an *A. euteiches*-infested field. Arbuscular mycorrhizal (AM)-fungi also have been shown to increase pea seedling emergence when inoculated with *A. euteiches* in greenhouse experiments (Bødker et al. 2002; Thygesen et al. 2004). Several studies indicated that solarization was essential for the control of root rot in temperate regions when combined with green manure crops, reduced dosages of chemicals, or biological control organisms (Katan 1987; Ramirez-Villapudua and Munnecke 1988).

1.4.5. Genetic resistance to *A. euteiches*

Utilization of pea cultivars with genetic resistance to *A. euteiches* would represent the most economic and effective management strategy for ARR in field pea. Unfortunately, fully or highly resistant pea cultivars are not available at present (Papavizas and Ayers 1974; Pfender et

al. 2001; Gaulin et al. 2007). Researchers have developed a number of pea breeding lines that are partially resistant or tolerant to *A. euteiches*, which could contribute to the prevention of yield losses in some pea producing regions (Davis et al. 1995; Pilet-Nayel et al. 2005; Hamon et al. 2011; Conner et at. 2013). Some pea genotypes used in host differential sets, such as Capella, MN 144, MN 313, MN 314, 902131, 90–2079, 552 and PI180693, have been reported to be partially resistant to different *A. euteiches* strains (Davis et al. 1995; Wicker and Rouxel 2001; Wicker et al. 2003). The genotypes PI 180693 and 552, in particular, have drawn considerable attention due to their high and stable partial resistance to ARR (Wicker et al. 2003; Pilet-Nayel et al. 2007). Conner et al. (2013) reported a high level of tolerance in the pea line 00-2067, with low disease severity, high vigor and good yield potential in an ARR disease nursery in Canada. This suggested that 00-2067 may be a promising source of resistance for introgression into agronomically desirable pea genotypes. Some sources of resistance, however, have been linked to undesirable traits for node-length and flower and hilum colors, which increase the difficulties associated with transferring this resistance to agriculturally-acceptable breeder pea lines (Marx et al. 1972). In addition, traditional phenotypic-based breeding for partial resistance has been constrained by the polygenic inheritance of resistance in field peas (Hamon et al. 2011). Therefore, the identification and mapping of minor genes for resistance is essential for breeding resistant pea lines.

Partial polygenic resistance is controlled by quantitative trait loci (QTLs) which have minor to major effects on plant pathogen suppression (Poland et al. 2009; Kou and Wang 2010). Several QTLs associated with partial resistance to *A. euteiches* have been identified using linkage mapping populations derived from crosses between two parental genotypes in a number of studies (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015; Desgroux et al. 2016). Three consistent QTLs, namely *Aph1*, *Aph2* and *Aph3*, were identified in a recombinant inbred line (RIL) population derived from Puget × 90-2079 and located on LGIVb, V and Ia (Pilet-Nayel et al. 2002); *Aph1* and *Aph3* were shown to be associated with partial resistance to both American and French strains of *A. euteiches*, while *Aph2* was resistant only to the French strain (Pilet-Nayel et al. 2005). Hamon et al. (2011) reported 135 additive-effect QTLs corresponding to 23 genomic regions and 13 significant epistatic interactions associated with partial resistance to *A. euteiches*. These QTLs were identified in two RIL populations from the crosses Baccara × PI 180693 and Baccara × 552 based. Five consistent genomic regions (*Ae-Ps1.2*, *Ae-Ps2.2*, *Ae-Ps3.1*, *Ae-Ps4.1* and *Ae-Ps7.6*) in two RIL populations affecting and between DSI and ADI were identified on LGI, II, III, IV and VII; *Ae-Ps1.2* was co-localized to *Aph3* identified by Pilet-Nayel et al. (2002). Hamon et al. (2013) used QTL meta-analysis to examine three previously described RIL populations derived from Puget × 90–2079 (Pilet-Nayel et al. 2002), Baccara × PI180693 and Baccara × 552 (Hamon et al. 2011), and a new one derived from DSP × 90–2131. They identified 27 meta-QTLs based on disease severity that were well distributed (three or four meta-QTLs per LG) over seven linkage groups (LG), and 11 of 27

meta-QTLs corresponding to 10 genomic regions were consistent (Hamon et al. 2013). Lavaud et al. (2015) also identified two major QTLs, *Ae-Ps4.5* and *Ae-Ps7.6*, and some minor QTLs in near-isogenic lines (NILs) from crosses with the resistant parental genotypes, 90-2131, PI180693 and 552. In a subsequent study, Lavaud et al. (2016) examined the function of *Ae-Ps4.5*, *Ae-Ps7.6* and some other minor QTLs and reported a significant effect of those QTLs on the prevention or decrease of ARR symptoms and pea root colonization by *A. euteiches*. Simple sequence repeat (SSR) markers developed by Loridon et al. (2005) were applied widely in the above studies as major screening or reference markers. In addition to SSR markers, various other molecular markers also have been used, including amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD) markers, inter simple sequence repeats (ISSRs), and sequence tagged sites (STSs). In recent years, the rapid development of single-nucleotide polymorphism (SNP) markers from whole genome sequencing of pea lines, along with decreasing genotyping costs, has led to the emergence of genome-wide association study (GWAS) as the standard approach to detect natural variations underlying complex traits, especially polygenic resistance to major diseases in legumes (Bao et al. 2014; Cheng et al. 2015).

Compared with linkage mapping analysis between resistant and susceptible genotypes, GWAS enables the analysis of wider genetic diversity, higher recombination rates due to the evolutionary history of the species, and thus can refine genomic regions associated with trait

variations substantially. Desgroux et al. (2016) conducted GWAS mapping with 13,204 SNPs to narrow down the confidence intervals (CIs) of QTLs associated with root rot severity in pea. In their study, 52 QTLs of small size-intervals were detected, which were more valuable than the QTLs with large size-intervals identified in previous studies, for increasing the levels of partial resistance to *A. euteiches* (Desgroux et al. 2016).

1.5 Research problems and objectives

Canada is the largest producer of field pea worldwide, and the cultivation of pea in this country has increased continuously in recent years (FAOSTAT 2017). Pea production, however, is limited by ARR, a severe soil-borne disease. Studies on pathogenic variability in *A. euteiches* have been disconnected and inconsistent. Given the absence of highly resistant pea genotypes, it can be difficult to develop standards to distinguish races of *A. euteiches*, although strain diversity has been examined via comparisons of pathogenic variability. Traditional cultural practices, such as crop rotation, have had limited utility in the management of ARR. Seed treatments have had no impact on reducing ARR through the whole life of the field pea plant, and few fungicidal seed treatments are available in Canada. While many major-effect QTLs have been identified through various molecular technologies, providing valuable resources for resistance pyramiding in pea breeding programs, partially resistant or tolerant pea genotypes adapted to the Canadian environment are still needed.

This Master's project was aimed at evaluating the extent of the ARR problem in Alberta, while evaluating various methods to manage this disease in an integrated manner. As such, the objectives of this work were to: (1) investigate the incidence and severity of ARR of pea field in Alberta; (2) examine the disease severity and yield loss of pea associated with different inoculum densities; (3) evaluate the effects of fungicide seed treatments on improving seedling emergence in *A. euteiches*-infested fields; (4) evaluate the resistance of pea cultivars to ARR in Alberta; and (5) identify molecular markers associated with *A. euteiches* resistance in a RIL population derived from a cross of 00-2067 × Reward.

1.6 References

Abbo, S., Rachamim, E., Zehavi, Y., Zezak, I., Lev-Yadun, S. and Gopher, A. 2011. Experimental growing of wild pea in Israel and its bearing on Near Eastern plant domestication. Ann. Bot. **107:** 1399-1404.

Agriculture and Agri-Food Canada 2017. Crop Profile for Field Pea in Canada, 2015. 3rd edition. A118-10/3-2015E-PDF

Alexopoulos, C. J. and Mims, C. W. 1979. Introductory Mycology. 3rd edition. 145-188 pp. New York, Chichester, Brisbane, Toronto.

Ali-Khan, S. T. and Zimmer, R. C. 1989. Production of field peas in Canada. Agriculture Canada. Publication 1710/E

Allmaras, R. R., Fritz, V. A., Pfleger, F. L. and Copeland, S. M. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil Tillage Res. **70:** 41-52.

Anonymous. 1996. Alberta special crop survey. 1995. Alberta Agriculture, Food and Rural Development. Market Analysis and Statistics Branch. pp. 21.

Armstrong-Cho, C., Tetreault, M., Banniza, S., Bhadauria, V. and Morrall, R. A. A. 2014. Reports of *Aphanomyces euteiches* in Saskatchewan. Can. Plant Dis. Surv. **94:** 193–194.

Badreddine, I., Lafitte, C., Heux, L., Skandalis, N. Spanou, Z., Martinez, Y., Esquerré-Tugayé, M.T., Bulone, V., Dumas, B. and Bottin, A. 2008. Cell wall chitosaccharides are

essential components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches.* Eukaryot Cell **7:** 1980-1993.

Bailey, K. L., Gossen, B.D., Gugel, R.k. and Morrall, R.A.A. 2003. Diseases of field crops in Canada. Canadian Phytopathological Society, University Extension Press, University of Saskatchewan, Saskatoon, SK.

Bao, Y., Vuong, T., Meinhardt, C., Tiffin, P., Denny, R., Chen, S., Nguyen, H. T., Orf, J. H. and Young, N. D. 2014. Potential of association mapping and genomic selection to explore PI 88788 derived soybean cyst nematode resistance. Plant Genome **7:** 1-13. doi:10.3835/plantgenome2013.11.0039.

Basu, P. K., Brown-CreÃte, R., Gourley, C. O., Haas, J. H., Harper, F. R., Lawrence, C. H., Seaman, W. L., Toms, H. N. W., Wong, S. I. and Zimmer, R. C. 1973. Prevalence and severity of diseases of processing peas in Canada, 1970-71. Can. Plant Dis. Surv. **53:** 49-57.

Beakes, G.W., Glockling, S.L. and Sekimoto, S. 2012. The evolutionary phylogeny of the oomycete "fungi". Protoplasma **249:** 3-19.

Bekkering E. 2011. Canadian Agriculture at a Glance: Pulse in Canada. Statistics Canada. Catalogue no. 96‑325‑X, No. 007. ISSN 0-662-35659-4

Beute, M.K. and Lockwood, J.L. 1967. Pathogenic variability in *Aphanomyces euteiches*. Phytopathology **57:** 57-60.

Bødker, L., Kjøller, R., Kristensen, K. and Rosendahl, S. 2002. Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza **12:** 7-12.

Bowers, J. H. and Parke, J. L. 1993. Epidemiology of Pythium damping-off and Aphanomyces root rot of pea after seed treatment with bacterial agents for biological control. Phytopathology **83:** 1466-1473.

Boys, C. A., Rowland, S.J., Gabor, M., Gabor, L., Marsh, I.B., Hum, S. and Callinan, R.B. 2012. Emergence of epizootic ulcerative syndrome in native fish of the Murray-Darling River System, Australia: hosts, distribution and possible vectors. PLoS One **7:** 1-10.

Bruin, G. C. A. and Edgington, L. V. 1983. Chemical control of diseases caused by zoosporic fungi: a many-sided problem. In Zoosporic plant pathogens: A modern perspective Buczacki, S. T. (ed.) London: Academic Press. pp. 193-223.

Burke, D. W. and Mitchell, J. E. 1968. Temperature and moisture effects on infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology (Abstract) **58:** 1045.

Burke, D. W., Mitchell, J. E. and Hagedorn, D. J. 1969. Selective conditions for infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology **59:** 1670-1674.

Carlson, L.E. 1965. Studies on the root rot of peas caused by *Aphanomyces euteiches*. Ph.D. thesis, University of Minnesota, Twin Cities, USA.

Chan, M. Y. K. and Close, R. C. 1987. Aphanomyces root rot of peas: Evaluation of methods for assessing inoculum density of *Aphanomyces euteiches* in soil. New Zeal. J. Agr. Res.**30:** 213-217.

Chang, K. F., Bowness, R., Hwang, S. F., Turnbull, G. D., Howard, R. J., Lopetinsky, K., Olson, M. and Bing, D. J. 2005. Pea diseases occurring in central Alberta in 2004. Can. Plant Dis. Surv. **85:** 89-90.

Chang, K. F., Hwang, S. F., Ahmed, H., Gossen, B., Turnbull, G. D. and Strelkov, S. 2013. Management strategies to reduce losses caused by fusarium seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288-289.

Cheng, P., Holdsworth, W., Ma, Y., Coyne, C.J., Mazourek, M., Grusak, M.A., Fuchs, S. and McGee, R. J. 2015. Association mapping of agronomic and quality traits in USDA pea single plant collection. Mol. Breed. **35**: 75.

Chupp, C. and Sherf, A. F. 1960. Vegetable diseases and their control. New York, Ronald Press Co. 434 pp.

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. 2013. Assessment of tolerance for reducing yield losses in field pea caused by *Aphanomyces* root rot. Can. J. Plant Sci. **93:** 473-482.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M. 2000. A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genet. Biol. **30:**17-32.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M. 2000. A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genet. Biol. **30:** 17-32.

Copeland, H. F. 1956. The classification of lower organisms. ix 302 pp., 45 fig. Frontis. Pacific Books, Palo Alto.

Daines, R. H. 1953. Capton – the new discovery in fruit fungicide. Fruit grower **73:**16.

Davey, C. V. and Papavizas, G. C. 1961. Aphanomyces root rot of peas as affected by organic and mineral soil amendments. Phytopathology **51:** 131-132.

Davis, D. W., Fritz, V. A., Pfleger, F. L., Percich, J. A. and Malvick, D. K. 1995. MN 144, MN 313 and MN 314: Garden pea lines resistant to root rot caused by *Aphanomyces euteiches* Drechs. HortSci. **30:** 639-640.

Desgroux, A., L'Anthoëne, V., Roux-Duparque, M., Rivière, J., Aubert, G., Tayeh, N. and Pilet-Nayel, M. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics 17: 124.

Diéguez-Uribeondo, J., García, M. A., Cerenius, L., Kozubíková, E., Ballesteros, I., Windels, C., Weiland, J., Kator, H., Söderhäll, K. and Martín, M.P. 2009. Phylogenetic relationships among plant and animal parasites, and saprotrophs in Aphanomyces (Oomycetes). Fungal Genet. Biol. **46:** 365-376.

FAOSTAT 2017. Food and Agriculture Organization of the United Nations Statistics. http://www.fao.org/faostat/en/#data/QC

Feng, J., Hwang, R., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E. and Xue, A. G. 2011. Identification of microsatellite markers linked to quantitative trait loci controlling resistance to Fusarium root rot in field pea. Can. J. Plant Sci. **91:** 199-204.

Feng, J., Hwang, R., Chang, K. F., Hwang, S. F., Strelkov, S. E., Gossen, B. D., Conner, R. L. and Turnbull, G. D. 2010. Genetic variation in *Fusarium avenaceum* causing root rot on field pea. Plant Pathol. **59:** 845-852.

Filipová, L., Petrusek, A., Matasová, K., Delaunay, C. and Grandjean, F. 2013. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in populations of the signal crayfish *Pacifastacus leniusculus* in France: Evaluating the threat to native crayfish. Plos One **8:** 1-10.

Fritz, V. A., Allmaras, R., Pfleger, F. L. and Davis, D. W. 1995. Oat residue and soil compaction influences on common root rot (*Aphanomyces euteiches*) of peas in a fine-textured soil. Plant Soil **171:** 235-244.

Garrett, S. D. 1944. Root disease fungi: A treatise on the epidemiology of soil-borne disease in crop plants, and a first exposition of the principles of root disease control. Massachusetts: Chronica Botanica p. 177.

Gaulin, E., Jacquet, C., Bottin, A. and Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol. Plant Path. **8:** 539-548.

Gaulin, E., Madoui, M.A., Bottin, A., Jacquet, C., Mathé, C., Couloux, A., Wincker, P. and Dumas, B. 2008. Transcriptome of *Aphanomyces euteiches*: new oomycete putative pathogenicity factors and metabolic pathways. PloS One **3:** e1723.

Gaűmann, E. A. 1952. The fungi. (Trans. By F.L. Wynd.) 420 pp., 440 figs. Hafner Publishing Co., New York

Gaűmann, E. A. 1963. Die Pilze. 541pp., 610 figs. Birkhaouser Verlag, Basel.

Gritton, E. T., Parke, J. L., Percich, J. A., Fritsz., Y. A., Kraft, J. M., Grau, C. R, Pfleger, F. L. and Allmaras, R. R. 1995. Integrated control of Aphanomyces root rot of pea (*Pisum sativum* L.). 2nd European Conference of Grain Legumes: Improving production and utilisation of grain legumes. pp. 148-149.

Grünwald N. J. 2003. The biology of the genus *Aphanomyces*. Proceedings of the 2nd International Aphanomyces Workshop. United States Department of Agriculture. 9-14 pp.

Guerriero, G., Avino, M., Zhou, Q., Fugelstad, J., Clergeot, P.-H. and Bulone, V. 2010. Chitin synthases from Saprolegnia are involved in tip growth and represent a potential target for antioomycete drugs. PLoS Pathog. **6:** e1001070.

Haenseler, C. M. 1926. Studies on the root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches* Drechsler. New Jersey Agricultural Experiment Station 46th Annual Report **46:** 467-484.

Hagedorn, D. J. 1984. Compendium of pea diseases / edited by Donald J. Hagedorn. *In:* Disease compendium series St. Paul, Minnisoda: American Phytopathological Society in cooperation with Deptartment of Plant Pathology, University of Wisconsin, Madison, 1984.

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V. and Lesne, A. 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theor. Appl. Genet. **123:** 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P. and Pilet-Nayel, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biol. **13:** 1-19.

Henig, R. M. 2000. The monk in the garden: how Gregor Mendel and his pea plants solved the mystery of inheritance. New York: Houghton Mifflin 2000.

Heyman, F., Lindahl, B., Persson, L., Wikstrom, M. and Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea. Soil Biol. Biochem. **39:** 2222-2229.

Hoch, H. C. and Mitchell, J. E. 1973. The effects of osmotic water potentials on *Aphanomyces euteiches* during zoosporogenesis. Can. J. Bot. **51:** 413-420.

Hossain, S., Bergkvist, G., Berglund, K., Glinwood, R., Kabouw, P., Mårtensson, A. and Persson, P. 2014. Concentration- and time-dependent effects of isothiocyanates produced from Brassicaceae shoot tissues on the pea root rot pathogen *Aphanomyces euteiches*. J. Agric. Food Chem. **62:** 4584-4591.

Hwang, S. F., Lopetinsky, K. and Evans, I. R. 1991. Effects of seed infection by *Ascochyta* spp., fungicide seed treatment, and cultivar on yield parameters of field pea under field conditions. Can. Plant Dis. Surv. **71:** 169-172.

Hwang, S.F. and Chang, K.F. 1989. Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69:** 139-141

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D., Howard, R. J. and Blade, S. F. 2003. Etiology, impact and control of rhizoctonia seedling blight and root rot of chickpea on the Canadian Prairies. Can. Plant Dis. Surv. **83:** 959-967.

Jean L., W., F.L., P., Vincent A., F. and R.R., A. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Plant and soil **188:** 43–48

Jermyn, W. A., Banfield, R. A., Hedley, J. and Russell, A. C. 1982. Effect of seed treatment on Aphanomyces root rot of peas. Proceedings of the Agronomy Society of New Zealand **12:** 15-18.

Jones, F. R. and Drechsler, C. 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agr. Res. **30:** 293-325.

Jones, F.R. and Linford, M.B. 1925. Pea disease survey in Wisconsin. Wisconsin Agriculture Experimental Station Research **64:** 1-34.

Katan, J. 1987. Soil solarization. Pages 77-105 *In:* Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York. 372 pp.

Karin, T., John, L. and Lars, B. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. Eur. J. Plant Path. **110:** 411-419.

Kerr, A. 1964. Influence of soil moisture on infection of peas by *Pythium ultimum.* Aust. J. Biol. Sci. **17:** 676-685.

King, E. B. and Parke, J. L. 1993. Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis. **77:** 1185-1188.

King, T. H. and Bissonnette, H. L. 1954. Physiologic specialization in *Aphanomyces euteiches* (Abstract). Phytopathology **44:** 495.

Knott, C. M. 1987. A key for stages of development of the pea (*Pisum sativum*). Ann. Appl. Biol. **111:** 233-245.

Kotova, V. V. and Tsvetkova, N. A. 1980. Effectiveness of chemical control measures against Aphanomyces root rot of peas. Rev. Plant Pathol. (Abstract) **59:** 342.

Kou, Y. J. and Wang, S. P. 2010. Broad-spectrum and durability: understanding of quantitative disease resistance. Curr. Opin. Plant Biol. **13:** 181-185.

Kraft, J. M. and Kaiser, W. J. 1993. *In:* Breeding for Stress Tolerance in Cool-Season Food Legumes. *In:* K. B. Singh and M. C. Saxena (eds.). Chichester, UK: John Wiley and Sons. pp. 123-144.

Kraft, J. M., Marcinkowsha, J. and Muehlbauer, F. J. 1990. Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet-sieving/baiting technique. Plant Dis. **74:** 716-718.

Kroon, L.P.N.M., Brouwer, H., de Cock, A.W. A. M. and Govers, F. 2012. The genus Phytophthora anno 2012. Phytopathology **102:** 348-364.

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D. and Turkington, T. K., 2002. Managing plant disease risk in diversified cropping systems. Agron. J. **94:** 198-209.

Lavaud, C., Baviere, M., Le Roy, G., Hervé, M. R., Moussart, A., Delourme, R. and Pilet-Nayel, M. 2016. Single and multiple resistance QTL delay symptom appearance and slow

down root colonization by *Aphanomyces euteiches* in pea near isogenic lines. BMC Plant Biol. **16:** 166.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. App. Genet. **128:** 2273-2288.

Lawson, H. M. and Topham, P. B. 1985. Competition between annual weeds and vining peas grown at a range of population densities: Effects on the weeds. Weed Res. **25:** 221-229.

Leclerc, M. C., Guillot, J. and Deville, M. 2000. Taxonomic and phylogenetic analysis of Saprolegniaceae (Oomycetes) inferred from LSU rDNA and ITS sequence comparisons. Antonie van Leeuwenhoek **77:** 369-377.

Leipe, D. D., Wainright, P. O., Gunderson, J. H., Porter, D., Patterson, D. J., Valois, F., Himmerich, S. and Sogin M. L. 1994. The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. Phycologia **33:** 369-377.

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-He`naut, I. and Burstin, J. 2005. Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor. App. Genet. **111:** 1022-1031.

Malvick, D. K. and Grau, C. R. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Dis. **85:** 740-744.

Malvick, D. K. and Percich, J.A. 1998a. Genotypic and pathogenic diversity among pea-infecting strains of *Aphanomyces euteiches* from Central and Western United States. Phytopathology **88:** 915-921.

Malvick, D. K. and Percich, J.A. 1998b. Variation in pathogenicity and genotype among single-zoospore strains of *Aphanomyces euteiches*. Phytopathology **88:**52-57.

Malvick, D.K. and Percich, J.A. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Dis. **83:** 51–54.

Manning, M. and Menzies, S.A. 1984. Pathogenic variability in isolates of *Aphanomyces euteiches* from New Zealand soils. New Zeal. J. Agr. Res. **27:** 569-574.

Marx, G. A., Schroeder, W. T., Provvidenti, R. and Mishance, W. 1972. A genetic study of tolerance in pea (*Pisum sativum* L.) to Aphanomyces root rot. J. Am. Soc. Hortic. Sci. **97:** 619-621.

Matari, N.H. and Blair, J.E. 2014. A multilocus timescale for oomycete evolution estimated under three distinct molecular clock models. BMC Evol. Biol. **14:** 1-21.

Mélida, H., Sandoval-Sierra, J. V, Diéguez-Uribeondo, J. and Bulone, V. 2013. Analyses of extracellular carbohydrates in oomycetes unveil the existence of three different cell wall types. Eukaryote Cell **12:** 194-203.

Meisrimler, C., Wienkoop, S., Lyon, D., Geilfus, C. and Lüthje, S. 2016. Long-term iron deficiency: Tracing changes in the proteome of different pea (*Pisum sativum* L.) cultivars. J. Proteomics **140:** 13-23. doi:10.1016/j.jprot.2016.03.024

Mitchell, J. E. and Yang, C. Y. 1966. Factors affecting growth and development of *Aphanomyces euteiches*. Phytopathology **56:** 917-922.

Moussart, A., Onfroy, C., Lesne, A., Esquibet, M., Grenier, E. and Tivoli, B. 2007. Host status and reaction of *Medicago truncatula* accessions to infection by three major pathogens of pea (*Pisum sativum*) and alfalfa (*Medicago sativa*). Eur. J. Plant Pathol. **117:** 57-69.

Muehlchen, A M., Rand, R. E. and Parke, J. L. 1990. Evaluation of crucifer green manures for controlling Aphanomyces root rot of peas. Plant Dis.**74:** 651-654.

Nars, A., Lafitte, C., Chabaud, M., Drouillard, S., Mélida, H., Danoun, S. and Bottin, A 2013. *Aphanomyces euteiches* cell wall fractions containing novel glucan chitosaccharides induce defense genes and nuclear calcium oscillations in the plant host *Medicago truncatula*. PLoS One **8:** 1-13.

Olofsson, J. 1967. Root rot of canning and freezing peas in Sweden. Acta Agr. Scand. **17:** 101-107.

Ondrej, M., Dostalova, R. and Trojan, R. 2008. Evaluation of virulence of *Fusarium solani* isolates on pea. Plant Prot. Sci. **44:** 9-18.

Oyarzun, P., Dijst, G., Zoon, F. and Maas, P. 1997. Comparison of soil receptivity to *Thielaviopsis basicola*, *Aphanomyces euteiches*, and *Fusarium solani* f. sp. *pisi* causing root rot in pea. Phytopathology **87:** 534-541.

Oyarzun, P., Gerlagh, M., Hoogland, A. and Vos, I. 1990. Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Nether. J. Plant Pathol. **96:** 301-311.

Papavizas, G. C. 1966. Suppression of Aphanomyces root rot of peas by cruciferous soil amendments. Phytopathology **56:** 1071-1075.

Papavizas, G. C. 1967. Comparison of treatments suggested for control of Aphanomyces root rot of peas. Plant Dis. Rep. **51:** 125-129.

Papavizas, G. C. and Lewis, J. A. 1971. Effect of amendments and fungicides on Aphanomyces root rot on peas. Phytopathology **61:** 215-220.

Papavizas, G.C. and Ayers, W.A. 1974. *Aphanomyces* species and their root diseases on pea and sugarbeet. US Dept. Agric. Agric. Res. Serv. Tech. Bull. 1485, 158pp.

Parke, J. L., Rand, R. E., Joy, A. B. and King, E. B. 1991. Biological control of Pythium damping-off and Aphanomyces root rot of peas by application of *Pseudomonas cepacia* or *P. fluorescens* to seed. Plant Dis. **75:** 987-992.

Paul, L. 1986. Disease of field pea (*Pisum sativum* L.) in the Peace River region of Alberta. M.Sc. Thesis, University of Alberta. p. 127.

Pfender, W. F. 1984. Aphanomyces root rot. *In:* D. J. Hagedorn (ed.), Compendium of Pea Diseases, St. Paul, MN, USA: APS Press. pp. 25-28.

Pfender, W. F. and Hagedorn, D. J. 1983. Disease progress and yield loss in *Aphanomyces* root rot of peas. Phytopathology **73**: 1109-1113.

Pfender, W., Malvick, D. K., Pfleger, F. L. and Grau, C. R. 2001. Aphanomyces root rot. Pages 9-13. *In:* J. M. Kraft and F. L. Pfleger (eds.) Compendium of pea diseases and pests. 2nd ed. APS Press, St. Paul, MN.

Pilet-Nayel M. L., Coyne C., Hamon C., Lesne´ A., Le Goff I., Esnault R., Lecointe R., Roux-Duparque M., McGee R., Mangin P., McPhee K., Moussart A. and Baranger A. 2007. Understanding genetics of partial resistance to Aphanomyces root rot in pea for new breeding prospects. *In:* Third International Aphanomyces workshop on legumes, 07–09 November 2007, Rennes, France, p. 36.

Pilet-Nayel, M. -L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2002. Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. Theor. Appl. Genet. **106:** 28-39.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology **95:** 1287-1293.

Poland, J., A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C. and Nelson, R. J. 2009. Shades of gray: The world of quantitative disease resistance. Trends Plant Sci. **14:** 21-29.

Reiling, T. P., King, T. H. and Fields, R. W. 1960. Soil indexing for pea root rot and the effect of root rot on yield. Phytopathology **50:** 287-290

Royo, F. Andersson, G., Bangyeekhun, E., Múzquiz, J. L., Söderhäll, K. and Cerenius, L. 2004. Physiological and genetic characterisation of some new *Aphanomyces* strains isolated from freshwater crayfish. Vet. Microbiol. **104:** 103-112.

Sauvage, H., Moussart, A., Bois, F., Tivoli, B., Barray, S. and Laval, K. 2007. Development of a molecular method to detect and quantify *Aphanomyces euteiches* in soil. FEMS Microbiol. Lett. **273:** 64-69.

Scharen, A. L. 1960. Germination of oospores of *Aphanomyces euteiches* embedded in plant debris. Phytopathology **50:** 274-277.

Schrum, H., Kotcon, J. and Verlinden, S. 2008. Organic Methods for Control of Root Rot in Pea and Spinach in Northeastern U.S., 16th IFOAM Organic World Congress, Modena, Italy, June 16-20, 2008. pp. 624-627.

Scott, W. W. 1961. A monograph of the genus Aphanomyces. Virginia Agr. Exp. Sta. Tech. Bull. **151:** 1-95.

Sekizaki, H., Yokosawa, R., Chinen, C., Adachi, H. and Yamane, Y. 1993. Studies on zoospore attracting activity: Synthesis of isoflavones and their attracting activity to *Aphanomyces euteiches* zoospore. Biol. Pharm. Bull. **16:** 698-701.

Shaffer, R. L. 1975. The major groups of Basidiomycetes. Mycologia **67:** 1-18.

Sherwood, R. T. and Hagedorn, D. J. 1958. Determining the common root rot potential of pea fields. *In:* Bulletin / University of Wisconsin. Agricultural Experiment Station; 531.

Sherwood, R. T. and Hagedorn, D. J. 1962. Studies on the biology of *Aphanomyces euteiches*. Phytopathology **52:** 150-154.

Slinkard, A. E., van Kessel, C., Feindei, D. E., Aii-Khan, S. T. and Park, R. 1994. Addressing farmers' constraints through on-farm research: peas in western Canada. *In:* F. J. Muehibauer and W. J. Kaiser (eds.) Expanding the production and use of cool season food legumes. Kluwer Academic Publisher. The Netherlands. pp. 877-889.

Smith, P. G. and Walker, J. C. 1941. Certain environmental and nutritional factor affecting Aphanomyces root rot of garden pea. J. Agric. Res. **63:** 1-20.

Sparrow, F.K. 1976. A developmental study of *Achlya recurve* utilizing light and electron microscopy. M.S. Thesis. University of Texas at El Paso. 121 pp.

Sundheim, L. 1972. Physiologic specialization in *Aphanomyces euteiches*. Physiol. Plant Pathol. **3:** 301-306.

Sundheim, L. and Wiggen, K. 1972. *Aphanomyces euteiches* on peas in Norway, isolation technique, physiologic races and soil indexing. Norgres Landbr. Hoiskoles Meld. **51:** 1-17.

Temp, M. V. and Hagedorn, D. J. 1967. Influence of cropping practices on Aphanomyces root rot potential of Wisconsin pea fields. Phytopathology **57:** 667-670.

Thygesen, K., Larsen, J. and Bodker, L. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. Eur. J. Plant Pathol. **110:** 411-419.

Tu, J. C. 1987. Integrated control of the pea root rot diseases complex in Ontario. Plant Dis. **71:** 9-13.

Tu, J. C. 1990. Effect of soil pH on pea root rots, yield and soil biology. Mededelingen Faculteit Landbouwwetenschappen **55:** 827-834.

Tu, J. C. 1992. Management of root rot diseases of peas, beans, and tomatoes. Can. J. Plant Pathol. **14:** 92-99.

Tu, J. C. 1994. Effects of soil compaction, temperature, and moisture on the development of the Fusarium root rot complex of pea in southwestern Ontario. Phytoprotection **75:** 125-131.

Tu, J. C. and Findlay, W. I. 1986. The effects of different green manure crops and tillage practices on pea root rots. Proceedings of British Crop Protection Conference. **3:** 229-236.

Uzuhashi, S., Kakishima, M. and Tojo, M. 2010. Phylogeny of the genus *Pythium* and description of a new genera. Mycoscience **51:** 337-365.

Vandemark, G. J., Barker, B. M. and Gritsenko, M. A. 2002. Quantifying *Aphanomyces euteiches* in alfalfa with a fluorescent polymerase chain reaction assay. Phytopathology **92:** 265-272.

Vandemark, G., Kraft, J.M., Larsen, R., Gritsenko, M. and Boge, W. 2000. A PCR based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. Phytopathology **90:** 1137-1144.

Voglmayr, H. and Riethmüller, A. 2006. Phylogenetic relationships of *Albugo* species (white blister rusts) based on LSU rDNA sequence and oospore data. Mycol. Res. **110:** 75-85.

Wakelin, S. A. 2001. Biological control of *Aphanomyces euteiches* root rot of pea with spore-forming bacteria. Ph.D. Thesis, Lincoln University, Christchurch, New Zealand.

Wakelin, S., Walter, M., Jaspers, M. and Stewart, A. 2002. Biological control of *Aphanomyces euteiches* root-rot of pea with spore-forming bacteria. Australas. Plant Pathol. **31:** 401-407.

Wang, N. 2009. Quality of western Canadian peas. Canadian Grain Commission. p. 4.

Wicker E., Hullé M. and Rouxel F. 2001. Pathogenic characteristics of isolates of *Aphanomyces euteiches* recovered from pea in France. Plant Pathol. **50:** 433-442.

Wicker, E. and Rouxel, F. 2001. Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. Eur. J. Plant Pathol. **107:** 919-929.

Wicker, E., Moussart, A., Duparque, M. and Rouxel, 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. Eur. J. Plant Pathol. **109:** 47-60.

Williams-Woodward, J. L., Pfleger, F. L., Fritz, V. A. and Allmaras, R. R. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches.* Plant Soil **188:** 43-48.

Xi, K., Stephens, J. H. G. and Hwang, S. F. 1995. Dynamics of pea seed infection by *Pythium ultimum* and *Rhizoctonia solani*: Effects of inoculum density and temperature on seed rot and pre-emergence damping-off. Can. J. Plant Pathol. **17:** 19-24.

Xue, A. G. 2003a. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. Phytopathology **93:** 329-335.

Xue, A. G. 2003b. Efficacy of *Clonostachys rosea* strain ACM941 and fungicide seed treatments for controlling the root rot complex of field pea. Can. J. Plant Sci. **83:** 519-524.

Zohary, D. and Hopf, M. 1973. Domestication of pulses in the Old World. Science **182:** 887-894.

Table 1.1. Production (in tonnes) and area harvested (in hectare) of dry peas in the world in 1972, 1990 and 2014 (FAOSTAT, 2017)

World regions	1972		1990		2014	
	Product	Area harvested	Product	Area harvested	Product	Area harvested
Africa	317 368	434 633	266 621	502 807	662 466	723 533
Americas	265 758	229 683	525 863	350 838	4 406 475	1 980 059
Asia	2 839 814	3 104 299	2 276 785	1 822 853	2 359 256	2 344 660
Europe	506 8165	4 286 156	13 192 740	5 791 026	3 396 009	1 632 736
Oceania	72 833	45 099	375 447	335 787	361 917	250 952
World total	8 536 938	8 099 870	16 637 456	8 703 311	11 186 123	6 931 941
Canada	27 365	47 840	123 400	264 000	3 444 800	1 467 000

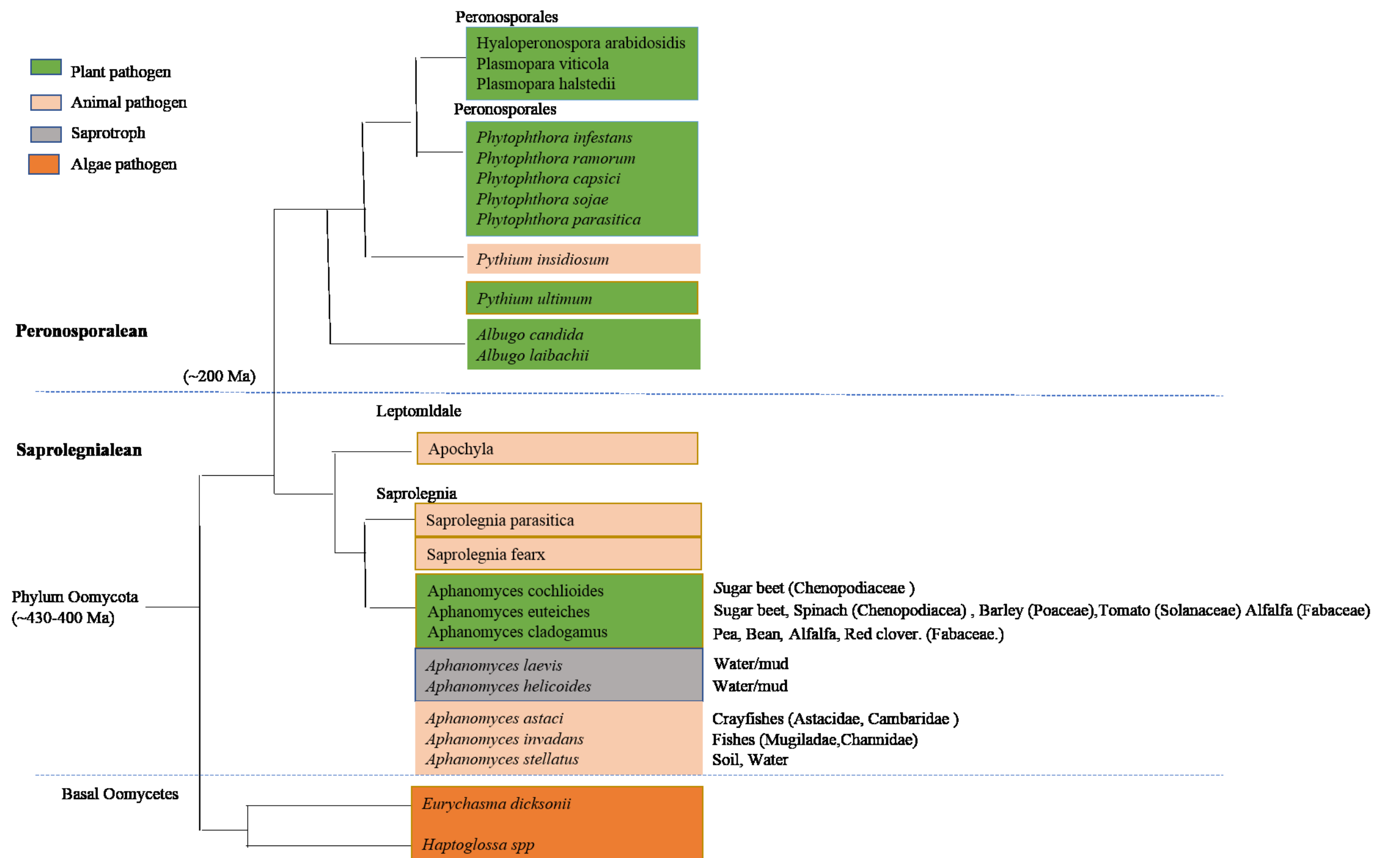

Fig. 1.1. Phylogenetic relationships of the main taxa of oomycetes, including the two major lineages: the Peronospolean lineage and the Saprolegnialean lineage. This graph was generated based on the descriptions of Beakes and Sekimoto (2012), Dieguez-Uribeondo et al. (2009) and Matari et al. (2014).

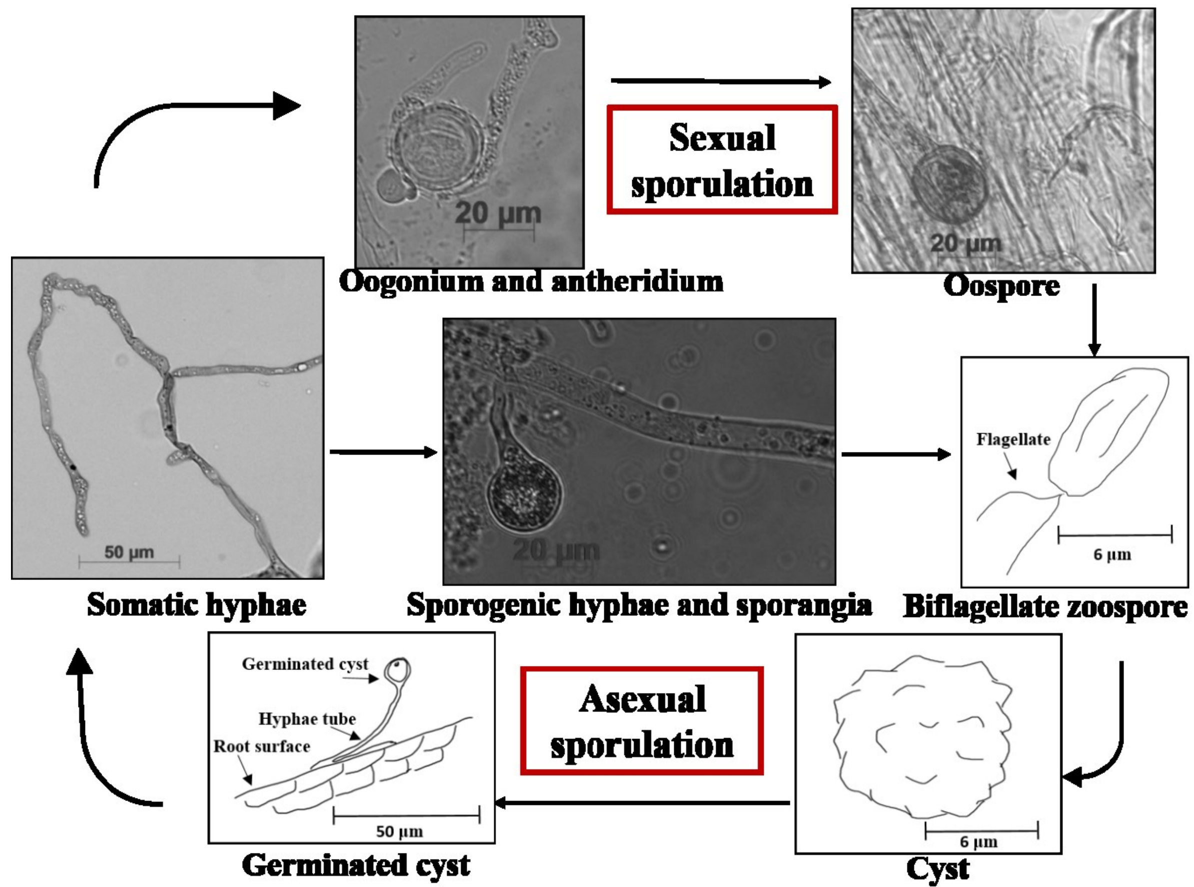

Fig. 1.2. The life cycle of *Aphanomyces euteiches* (modified from Gaulin et al. 2007 with images and illustrations by Longfei Wu).

Chapter 2: The occurrence of and microorganisms associated with root rot of field pea in Alberta in 2016[1]

2.1 Introduction

Field pea (*Pisum sativum*) is a valuable cash crop with high protein content and the ability to improve soil N balance (Hossain et al. 2014). Alberta is the second largest pea producing region in Canada (Bekkering 2011). The cultivation of pea, however, can be constrained by root rot, which is caused by a number of soilborne plant pathogens. In Alberta, these have included *Fusarium* spp. (Feng et al. 2010; Chang et al. 2013), *Phytophthora sansomeana* (Chang et al. 2017), *Rhizoctonia solani* (Hwang et al. 2003), and *Pythium* spp. (Laflamme 1998). Recently, however, root rot caused by *Aphanomyces euteiches* was reported in Alberta for the first time (Chatterton et al. 2015). This is a soilborne oomycete first identified nearly a century ago (Jones and Drechsler 1925), which can cause yield losses of up to 86% in heavily infected pea crops (Pfender and Hagedorn 1983). The current study was undertaken to gain a better understanding of the identity and occurrence of the microorganisms associated with root rot of field pea in Alberta, with a particular emphasis on *A. euteiches*.

2.2 Materials and methods

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[1]A version of this chapter has been published as: Wu, L. F., Chang, K. F., Fu, H., Akter, I., Li, N., Hwang, S. F., Tumbull, G. D. and Strelkov, S. E. 2017. The occurrence of and microorganisms associated with root rot of field pea in Alberta in 2016. Can. Plant Dis. Surv. 97: 193-195.

The occurrence and severity of root rot on field pea were investigated in a total of 71 commercial fields distributed across five counties in Alberta from July 8 - 24, 2016. Five randomly selected sites were surveyed in each crop in a 'W'- shaped sampling pattern. At each of the five sampling sites, 20 pea plants were chosen randomly and dug from the ground. The soil was carefully cleaned off from the root samples to preserve an intact root system. The percentage of symptomatic plants sampled within a field was recorded, while root rot severity was rated on scale of 0-4 (Chang et al. 2013). Ten pieces from each infected root sample were used to isolate the pathogens associated with the root rot complex, as described by Chang et al. (2005). The root pieces were transferred onto Petri dishes filled with potato dextrose agar (PDA) or selective metalaxyl-benomyl-vancomycin (MBV) medium (Pfender et al. 1984) for the isolation of *A. euteiches*.

2.3 Result and discussion

The distribution of root rot was uneven across the 71 pea crops surveyed (Table 2.1). The mean incidence of the disease was similar in the fields sampled at Edmonton, Drumheller and Sturgeon County, with an average of 69% ranging from 7 - 100%. Disease distribution was often patchy in specific fields and associated with wetter, low-lying areas (Fig. 2.1). At Vermillion and Westlock, root rot incidence was lower, with a mean of 41% ranging from 2 - 100%. Across all fields surveyed in Alberta, the mean disease incidence was 58%, while the average severity was 1.3 with a range of 0.01 - 3.4.

A total of 364 symptomatic root samples were cultured on PDA and MBV for pathogen isolation. Species of *Fusarium* were isolated most commonly from these roots, followed by *Pythium* spp., *A. euteiches* and *Rhizoctonia* spp. (Table 2.2). A mixture of *Fusarium* spp. and *Pythium* spp. was recovered from 67% of the roots, which suggested that an interaction between these two species frequently results in root rot. *Rhizoctonia* spp. was identified only from Sturgeon County at an incidence of 5%.

2.4 References

Bekkering E. 2011. Canadian Agriculture at a Glance: Pulse in Canada. Statistics Canada. Catalogue no. 96‑325‑X, No. 007. ISSN 0-662-35659-4

Chang, K. F., Bowness, R., Hwang, S. F., Turnbull, G. D., Howard, R. J., Lopetinsky, K., Olson, M. and Bing, D. J. 2005. Pea diseases in central Alberta in 2004. Can. Plant Dis. Surv. **85:** 89-90. (http://www. cps-scp.ca/cpds.htm)

Chang, K. F., Hwang, S. F., Ahmed, H. U., Gossen, B. D., Turnbull, G. D. and Strelkov, S. E. 2013. Management strategies to reduce losses caused by *Fusarium* seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chang, K., Hwang, S., Ahmed, H., Fu, H., Zhou, Q., Strelkov, S. and Turnbull, G. 2017. First report of *Phytophthora sansomeana* causing root rot in field pea in Alberta, Canada. Crop Protection **101:** 1-4. doi:10.1016/j.cropro.2017.07.008

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288-289.

Feng, J., Hwang, R., Chang, K. F., Hwang, S. F., Strelkov, S. E., Gossen, B. D., Conner, R. L. and Turnbull, G. D. 2010. Genetic variation in *Fusarium avenaceum* causing root rot on field pea. Plant Pathol. **59:** 413-421.

Hossain, S., Bergkvist, G., Berglund, K., Glinwood, R., Kabouw, P., Mårtensson, A. and Persson, P. 2014. Concentration- and time-dependent effects of isothiocyanates produced

from Brassicaceae shoot tissues on the pea root rot pathogen *Aphanomyces euteiches*. J. Agric. Food Chem. **62:** 4584-4591.

Hwang, S. F. and Chang, K. F. 1989. Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69:** 139-141.

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D., Howard, R. J. and Blade, S. F. 2003. Etiology, impact andcontrol of rhizoctonia seedling blight and root rot of chickpeaon the Canadian Prairies. Can. J. Plant Sci. **83:** 959-967.

Jones, F.R. and Drechsler, C. 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agri. Res. **30:** 293-325.

Laflamme, P. 1998. Diseases of field pea, *Pisum sativum* L., in the Peace River Region of Alberta. M.Sc. Thesis

Pfender, W. F. and Hagedorn, D. J. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology **73:** 1109-1113.

Pfender, W. F., Delwiche, P. A., Grau, C. R. and Hagedorn, D. J. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Dis. **68:** 845-847.

Pfender, W., Malvick, D. K., Pfleger, F. L. and Grau, C. R. 2001. Aphanomyces root rot. In J. M. Kraft and F. L. Pfleger (eds.) Compendium of pea diseases and pests. 2nd ed. APS Press, St. Paul, MN. pp. 9-13.

Table 2.1. Incidence and severity of pea root rot in Alberta in 2016.

County or municipality	No. of fields surveyed	Root rot incidence (%)		Root rot severity (0-4)*	
		Mean	**Range**	**Mean**	**Range**
Edmonton	5	63	7-100	1.4	0.9-2.5
Drumheller	2	69	38-100	1.8	0.1-3.0
Sturgeon	19	74	13-100	1.6	0.2-3.0
Vermillion	26	43	9-92	0.8	0.1-2.4
Westlock	19	39	2-100	0.9	0.01-3.4
Total/Average	71	57.6	2-100	1.3	0.01-3.4

*0 = healthy roots with no visible symptoms of root rot; 1 = slight water-soaking of the primary or secondary roots (1-25% discoloration); 2 = moderate water-soaking of the primary or secondary roots or epicotyls with light-brown areas and more extensive (26-50%) discoloration; 3 = infected areas extensive, soft, but the entire root is not collapsed, the epicotyl is not markedly shrivelled (51-75% discoloration); 4 = extensive discoloration of the roots with tissue collapse and disintegration, or plant completely dead (76-100% discoloration).

Table 2.2. Incidence (%) of the pathogens recovered from pea roots collected in Alberta in 2016 and showing symptoms of root rot.

County or municipality	No. roots tested	No. fields tested	*Fusarium* spp. (*F*)	*Pythium* spp. (*P*)	F+P	*Aphanomyces* euteiches*	*Rhizoctonia* spp.
Edmonton	39	3	97	92	90	5	0
Drumheller	18	2	100	44	44	6	0
Sturgeon	144	17	76	44	74	0	5
Vermillion	40	4	95	68	65	0	0
Westlock	123	15	85	62	60	5	0
Total/Avg.	364	41	90.6	62	66.6	3.2	1

*Data were obtained on the selective medium metalaxyl-benomyl-vancomycin (MBV) for the isolation of *Aphanomyces euteiches*.

Fig. 2.1. Field pea plants affected by severe root rot in a low-lying area of a field in Sturgeon County, Alberta.

Chapter 3: Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*

3.1 Introduction

Field pea is a valuable cash crop due to its high protein content and ability to improve soil fertility (Hossain et al. 2012). It is widely cultivated for human and livestock consumption. Field pea is adapted to temperate climates, but also grows well at high altitudes in the tropics and subtropics where the temperature is cool. Canada is the largest producer and exporter of field pea worldwide (Statistics Canada 2011). Unfortunately, pea cultivation is affected by the root rot pathogen, *Aphanomyces euteiches*. This oomycete is one of the most destructive soil-borne pathogens of legume crops. It has been reported from most regions where field peas are cultivated, including North America, Europe, Japan, Australia and New Zealand (Wade 1955; Yokosawa et al. 1974; Wicker and Rouxel 2001), and can cause yield losses as high as 86% (Pfender and Hagedorn 1983). Recently, *A. euteiches* was recorded in Alberta, Canada, for the first time, when it was identified in seven fields within a 200-km radius in the southern part of the province (Chatterton et al. 2015). Its relatively wide distribution led to the suggestion that *A. euteiches* likely had been present in Alberta for some years (Chatterton et al. 2015), a suggestion that was strengthened by the results of a root rot survey that found *A. euteiches* to be the second most commonly recovered pathogen after *Fusarium* spp. (Wu et al. 2017).

The spread and development of ARR requires high soil moisture and temperatures between 16-18°C (Papavizas and Ayers 1974). *Aphanomyces euteiches* can infect leguminous hosts at any stage of plant development, resulting in different symptoms (Gaulin et al. 2007). If infection occurs at the early growth stages, the roots of affected seedlings become soft and water-soaked with a honey-brown to blackish-brown discoloration. The pathogen spreads intercellularly through the cortical tissue, destroying the rootlets. As the plant grows, brown lesions and cortical decay of the lateral roots develop as a result of infection, limiting nutrient and water uptake from the soil (Papavizas and Ayers 1974). Eventually, the plants start to wilt, causing premature death and yield losses.

There are very few methods available for the management of ARR in field pea. The thick-walled oospores can persist in the soil for more than 10 years, and hence the recommended length of rotation between host and non-host crops is more than six years (Hossain et al. 2012). While the diversification of the cropping sequence can be an effective long-term strategy for the management of ARR (Krupinsky et al. 2002), the length of the rotations necessary may be unacceptable to most farmers. Alternatively, seed treatments have been evaluated to improve seedling emergence. For example, Xue (2003) demonstrated that in *A. euteiches*-infested fields, pea seeds treated with a combination of the biocontrol agent *Clonostachys rosea* (Link) Schroers strain ACM941 and several common fungicides had better germination rates than those coated only with the fungicides. Oyarzun et al. (1990) reported that seed treatment with the fungicide

Fosetyl-Al (aluminum tris) resulted in effective control of root rot caused by *A. euteiches* under greenhouse conditions. The coating of seeds with arbuscular mycorrhizal fungi also has been shown to increase pea seed emergence following inoculation with *A. euteiches* in greenhouse experiments (Bødker et al. 2002; Thygesen et al. 2004). In Canada, only INTEGO Solo (ethaboxam) seed treatment is registered in legumes for control of *Pythium* and suppression of seed rot caused by *Phytophthora* and *Aphanomyces*. Effective fungicidal or biological seed treatments for *Aphanomyces* control are still needed in Alberta.

Genetic resistance may offer an economic way of controlling *A. euteiches*, but completely resistant cultivars of field pea have not been identified (Pfender et al. 2001). Shehata et al. (1983) and Malvick and Percich (1999) reported tolerance to ARR in several plant introduction (PI) lines of pea. According to Conner et al. (2013), the pea line 00-2067 was tolerant to ARR, with higher yields and lower disease severity than other genotypes in soil infested with *A. euteiches*. Tolerant pea lines were reported to have smaller root lesions and produced fewer oospores in infected roots of pea seedlings, 8-days after zoospore inoculation, compared with susceptible cultivars in a greenhouse experiment (Kraft and Boge 1996). Marx et al. (1972) demonstrated that the tolerance to ARR is a genetic trait and several genetic mapping studies have identified quantitative trait loci (QTLs) that are linked to resistance in field pea (Pilet-Nayel et al. 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015).

The objectives of this study were to: (1) examine the effect of inoculum density on the

incidence and severity of ARR in field pea; (2) evaluate the efficacy of seed treatments on seedling emergence and plant health; and (3) evaluate the tolerance of some pea cultivars to *A. euteiches* isolates collected in Alberta.

3.2 Materials and methods

3.2.1 Preparation of inoculum

An isolate of *A. euteiches*, Ae-MRDC1, was obtained from pea roots planted in soil samples collected from a root rot disease nursery in Morden, Manitoba, in 2014. The isolation and purification of Ae-MRDC1 was carried out as Wicker et al. (2003). Briefly, the infected root tips from the soil samples were surface-sterilized in a 1% NaClO solution for 30 s, rinsed in sterilized water three times, and placed on metalaxyl-benomyl-vancomycin (MBV) medium (Pfender et al. 1984). After 2 days, colonies with the morphological characteristics of *A. euteiches* as per the keys of Dick (1973) and Scott (1961) were selected and transferred onto water agar medium for another two days. Single hyphal tips were cut from selected colonies under a stereomicroscope and transferred onto potato dextrose agar (PDA) for preservation. The disease severity indices caused by Ae-MRDC1 were > 1 on each of the differential pea genotypes of Wicker and Rouxel (2001) (‘Baccara’ = 2.3, ‘Capella’ = 1.9, ‘MN313’ = 2.7, ‘902131’ = 2.9, ‘552’ = 2.8, and ‘PI180693’ = 1.5) and, therefore, Ae-MRDC1 was classified as virulence pathotype I as defined by Wicker and Rouxel(2001).

The identity of Ae-MRDC1 and a culture of this isolate re-isolated from pea roots was confirmed by PCR analysis as *A. euteiches*. Briefly, total genomic DNA of the isolate was

extracted according to Vandemark et al. (2002) and the primers 136 Forward (5' GACTGCAATGTCGTCCAAGACTT 3') and 211 Reverse (3' AGCTAGAAGTAGAGTCGAAACA 5') were used to amplify a 76-bp fragment specific to *A. euteiches* (Vandemark et al. 2002; Sauvage et al. 2007). Amplification conditions consisted of 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s and extension at 72°C for 45 s. The products were resolved on a 1.5% agarose gel by electrophoresis for 1.5 h at 100 V (Vandemark et al. 2002).

Sand-oat based inoculum of *A. euteiches* was applied in field and greenhouse tests according to the protocol described by Papavizas and Ayers (1974). Briefly, rolled oats (500 mL sand, 150 g oats and 100 mL water per bag) were autoclaved and then inoculated with oospores of *A. euteiches*, followed by incubation for 4 weeks in darkness at 25°C. The grain inoculum was dried at room temperature and ground in a blender. To assess the viability of the sand-oat inoculum, serial dilutions were made with distilled water and the number of colony-forming units (CFUs) per gram of inoculum in the diluted samples was assessed on a selective medium (MBV agar) (Pfender et al. 1984). The concentration of the inoculum was adjusted to 1×10^5 CFU g^{-1} with sterile sand.

3.2.2 Plant materials

Plant material consisted of 22 field pea cultivars commonly grown in central Alberta. These were evaluated for their susceptibility to *A. euteiches* pathotype I using a seed inoculation

method. Briefly, the pea seeds were surface-sterilized in a 2% NaClO solution for 3 min, and rinsed three times in sterilized water. Five seeds of each cultivar were then placed in 9 cm-diameter Petri dishes filled with 20 mL PDA medium, on which four agar blocks colonized by *A. euteiches* also had been placed. The Petri dishes were incubated at 16°C in the dark. After 7 days, the number of seeds that had germinated and still had healthy roots was recorded. The experiment was arranged in a completely randomized design with four replications, and was repeated once. The two pea cultivars with the lowest germination rates, 'Abarth' and 'Horizon', were selected for the field and greenhouse experiments.

3.2.3 Field preparation

Field trials were conducted at two sites at the Crop Diversification Center North (CDCN), Alberta Agriculture and Forestry, Edmonton, AB (lat. 53˚34'N, long. 113˚31'W), in 2015 and 2016. Another field trial was conducted at the Agriculture and Agri-Food Canada (AAFC) Morden Research and Development Centre, Morden, MB (lat. 49°11' N, long. 98°5' W) in 2016. All field sites, both in Edmonton and Morden, were located on a black, chernozemic sandy loam soil. The Edmonton sites had been planted to canola (*Brassica napus* L.) in 2014 and to wheat (*Triticum aestivum* L.) in 2015. Field peas had been grown continuously in monoculture at the Morden site for the past 20 years and ARR was known to be severe. The field plots were sprayed with Pursuit (imazethapyr) or with Basagran Forte (bentazon), Assure II (quizalofop) and Pursuit on June 18, 2015, and June 13, 2016, at Edmonton and Morden, respectively, prior to planting to suppress the growth of weeds.

3.2.4 Inoculum density experiments

Field plots, each consisting of four 6-meter rows spaced 40 cm apart, were established on June 15, 2015, and May 17, 2016, at the two sites in Edmonton, AB, to determine the effect of inoculum density on seedling growth and productivity. Seeds of 'Abarth' and 'Horizon' were sown at a depth of 5 cm with a push seeder and mixed with 150 mL sand inoculum row $^{-1}$, as well as a commercial rhizobial inoculant (10 mL per 6-m row) (*Rhizobium leguminosarum* biovar *viciae*, a granular formulation supplied by Cell-Tech, Monsanto, Winnipeg, MB). Four concentrations of grain inoculum (0, 80, 160 and 240 CFU mL^{-1}) were applied. The trial was arranged in a two-factor randomized complete block design (RCBD) with four replications, of which the two fixed effects were cultivar and inoculum concentration. Emergence counts and seedling vigor (0 – 4, where: 0 = completely healthy; 1 = slightly wilted; 2 = moderately wilted; 3 = adverse wilt; 4 = completely wilt, plant dead) were recorded at 2 and 4 weeks after seeding, respectively, based on the methods described by Chang et al. (2013). Three months after seeding, root rot severity and root nodulation were recorded for 10 randomly selected plants from each plot as previously described. The plots were harvested on September 29, 2015, and September 7, 2016, and the seeds from each plot were weighed to determine yield.

In a greenhouse experiment, the pea cultivars 'Abarth' and 'Horizon' were sown in 600 mL plastic cups containing 400 mL of a steam-pasteurized potting mixture consisting of loam, sand, and vermiculite (3:1:1, vol:vol:vol), 100 mL *A. euteiches* inoculum, and 1 mL *R. leguminosarum*. The cups were maintained in a greenhouse at 26-28°C with a 12 h photoperiod,

and were watered daily in the morning and evening to ensure high moisture levels in the potting mixture. Ten seeds per cup (9 cm in diam and 10.5 cm in depth) were planted at a depth of 2 cm, and the sand-oat inoculum included 10 different concentrations of *A. euteiches* (1.03, 1.72, 2.42, 3.11, 3.80, 4.50, 5.14, 5.89, 6.58 ln of CFU mL^{-1} and a pathogen-free control). The cups were arranged in a two-factor RCBD with six replications (cups) per treatment, of which the two fixed effects were cultivar and inoculum concentration. The emergence rate, height and degree of vigor (0 – 4) of the seedlings were assessed at 7 days after seeding. The plants in each cup were uprooted four weeks after seeding and root rot severity and nodulation were evaluated. Nodulation was evaluated on a 0-4 scale as described by Chang et al. (2013). The experiment was conducted twice.

3.2.5 Fungicide seed treatment experiments

Field experiments were established on June 15, 2015, and May 17, 2016, at two sites at Edmonton, and on May 11, 2016, at one site at Morden, to evaluate the efficacy of five seed-treatment fungicides: Apron Advance (thiabendazole + fludioxonil + metalaxyl) +Vibrance (difenoconazole+ metalaxyl-M+ sedaxane), Intego Solo (ethaboxam), BAS 516F (boscalid + pyraclostrobin), BAS 720F (metalaxyl + pyraclostrobin + fluxapyroxad) and BAS 516F + BAS 720F (1:3). The experiments were arranged in a one-factor RCBD with four replications. The seed treatments were applied at the manufacturers' recommended rates (Table 3.1). In Edmonton, two fungicide-free controls also were planted in *A. euteiches*-non-inoculated and *A.*

euteiches-inoculated plots. Seeds of the susceptible 'Horizon' were treated with one of five fungicide formulations and sown at a depth of 5 cm along with 150 mL sand-oat inoculum (350 CFU mL^{-1}) per 6-m row spaced 40 cm apart. At Morden, seeds treated with the five fungicides and one fungicide-free control were sown in a field naturally infested with *A. euteiches*. The four row plots were trimmed to 5 m in length with 0.6 m spacing between the rows. Emergence rates, root rot severity, vigor and nodulation rates were recorded as in the inoculum density experiment. The field plots were hand-harvested on September 29, 2015, and September 7, 2016, at Edmonton and on August 15, 2016, at Morden. The seeds harvested from each plot were weighted to determine the yield.

In greenhouse experiments, the two susceptible pea cultivars 'Horizon' and 'Abarth', were treated with the same fungicides as above to assess their efficacy against ARR. The plants were grown in 600 mL plastic cups as described previously, at a density of 10 seeds per cup. The *A. euteiches* inoculum concentration was 350 CFU mL^{-1}. The treatments were arranged in a two-factor RCBD with six replications (cups) per treatment, of which the cultivar and seed treatment were fixed effects. The entire experiment was repeated once. Emergence rate, plant height and seedling vigor, root rot severity and nodulation were recorded as previously described.

3.2.6 Evaluation of host resistance

A total of 22 field pea genotypes were evaluated for their response to *A. euteiches* under field conditions at the Edmonton sites in 2015 and 2016. Treatments were arranged in a randomized

split-plot design with four replications, in which the main plots consisted of inoculated and non-inoculated sites, while the sub-plots comprised the different pea genotypes. The 80 seeds were sown in the plots in four 6-meter rows spaced 40 cm apart at a depth of 5 cm on May 12, 2015, and May 4, 2016. At Edmonton, sand-oat based inoculum of *A. euteiches* (350 CFUs mL^{-1}) was applied with the seeds at a rate of 150 mL per 6 m row. Emergence rates, root rot severity, vigor, nodulation and yield were measured as described above for the inoculum density test. The plots were harvested by small plot combine on September 23, 2015, and September 8-9, 2016, and yields determined.

3.2.7 Disease rating

Plants in the greenhouse and field studies were uprooted carefully from the soil 3-4 weeks after seeding. The root samples were washed with tap water and examined for symptoms of root rot. The roots of each plant in an experimental unit were rated on the disease severity of Papavizas and Ayers (1974), where: 0 = healthy roots with no visible symptoms of root rot; 1 = slight water-soaking of the primary or secondary roots (1-25% discoloration); 2 = moderate water-soaking of the primary or secondary roots or epicotyls with light-brown areas and more extensive (26-50%) discoloration; 3 = infected areas extensive, soft, but the entire root is not collapsed, the epicotyl is not markedly shrivelled (51-75% discoloration); 4 = extensive discoloration of the roots with tissue collapse and disintegration, or plant completely dead (76-

100% discoloration). A disease severity index (DSI) was calculated by averaging the root rot severity of each experimental unit (Papavizas and Ayers, 1974).

3.2.8 Statistical analysis

All statistical analyses were conducted using R v. 3.2.3 software (R Core Team, 2015). Data were tested for normality using Lilliefors (Kolmogorov-Smirnov) normality test, and Bartlett's test was used to assess the homogeneity of the variance among trials. Each data set was assessed using a general linear model for the analysis of variance, and the least square means were compared using least significant differences (LSD) at $P < 0.05$. In greenhouse experiments, where repetition × treatment was not significant, the data were pooled across repetitions for the analysis. In field experiments, the data from the inoculum density test, seed treatment test and resistant cultivar evaluation were analyzed separately by year because the year × treatment interactions were significant ($P < 0.05$). Linear regressions were carried out to determine the relationship of inoculum density with root rot severity, plant height, root nodulation, degree of wilting (vigor), and emergence rates in the greenhouse. In the inoculum density study in the greenhouse and field, and seed treatment study in the greenhouse, the difference between 'Abarth' and 'Horizon' was not significant ($P < 0.05$). Therefore, data from these cultivars were pooled.

A mixed ANOVA model was used to analyze the data from the field trials, using inoculum concentrations in the inoculum density test, fungicides in the seed treatment trial, and pea

genotypes in the resistance evaluation test as the fixed factors, while years, sites and blocks were treated as random factors. Least significant difference comparisons were used to determine if the analyzed traits differed among treatments. When the results of the ANOVA were significant ($P < 0.01$), linear response and regression analyses were run to examine the relationships among the treatments and host genotypes. Pearson correlation coefficient analysis was conducted to examine the correlations among the parameters in the seed treatment study in the greenhouse. A genotype plus genotype-by-environment (GGE) biplot analysis (Yan and Kang 2002) was conducted to investigate the stability of resistance to *A. euteiches* and the yield performance of the pea genotypes over the field trials.

3.3 Results

3.3.1 Inoculum density

In the greenhouse tests, the effects of inoculum concentration on plant height, root rot severity and root nodulation were significant ($P < 0.05$). The ANOVA revealed a significant effect of inoculum concentration ($P < 0.05$). As the inoculum density increased, pea root rot became more severe, resulting in reduced plant height and root nodulation (Fig. 3.1a, b and c). The logarithmic linear regression model that explained the relationship between the inoculum concentration and root rot severity was: $y = 0.799 \ln(x) – 0.983$, $R^2 = 0.90$ (Fig. 3.1a). In the case of inoculum concentration and plant height, the equation was: $y = -6.296 \ln (x) + 119.7$, $R^2 = 0.67$ (Fig. 3.1b), while between inoculum concentration and root nodulation it was: $y = - 0.454 \ln (x) + 2.802$, $R^2 = 0.90$ (Fig. 3.1c). The Pearson correlation analysis showed a negative

relationship between root rot severity and nodulation (Fig. 3.1d). A linear regression model between disease severity and nodulation was: y = -0.549x + 2.205 (R^2 = 0.76).

Rainfall was light in 2015 with major showers occurring only in mid-June, July and September (data not shown). Frequent, and sometimes heavy, rainfall events occurred throughout the growing season in 2016. As a result, weather conditions were more conducive for ARR development in 2016 than in 2015. The year × treatment interaction was significant. Therefore, the data were analyzed separately by year for all of the field trials. The values for disease severity, emergence rate, vigor and yield were consistently greater in 2016 than in 2015 across all inoculum concentrations (Fig. 3.2). Under field conditions, all of the inoculated treatments had a higher ARR severity and significantly lower pea seedling emergence, vigor and yield compared with the non-inoculated control (Fig. 3.2). Emergence and seedling vigor decreased with increased in inoculum concentration. A linear relationship was observed between inoculum concentration and root rot severity, emergence rate, vigor and yield in both 2015 and 2016. The models that described the relationship of inoculum concentration with root rot severity, seedling emergence, vigor and yield were: y = 0.186x + 0.797, R^2 = 0.88; y = -1.8595x + 76.406, R^2 = 0.92; y = -0.140x + 3.010, R^2 = 0.92; and y = -1.1833x + 110.8, R^2 = 0.60, respectively, in 2015. In 2016, the models were: y = 0.1635x + 1.262, R^2 = 0.68; y = -0.411x + 65.683, R^2 = 0.57; y = -0.249x + 3.872, R^2 = 0.97; and y = -6.45x + 289.49, R^2 = 0.73, respectively.

3.3.2 Fungicide seed treatment

In the field experiment at all sites in both 2015 and 2016, symptoms of root rot appeared as a brown discolouration of the roots at 4 weeks after seeding. None of the treatments, however, significantly improved seedling vigor and yield, or reduced disease severity, compared with the inoculated control at either Edmonton or Morden (Tables 3.2 and 3.3). The fungicide treatment Apron Advance + Vibrance significantly increased root nodulation in 2015 at both of the Edmonton sites, but other fungicide effects were not significant at any of the sites in 2016.

In the greenhouse study, the differences between inoculated and non-inoculated controls for plant height, disease severity, root nodulation and vigor were significant (Table 3.4). The Pearson correlation coefficient analysis indicated that disease severity was correlated negatively with plant height, root nodulation and vigor (Table 3.5). All five fungicides except Apron Advance + Vibrance and BAS 516 + BAS 720 increased plant height, root nodulation and vigor, and reduced disease severity significantly, compared with the inoculated control ($P < 0.05$) (Table 3.4). Seed treatment with Intego Solo resulted in the greatest mean height, vigor and nodulation, while seed treatment with BAS 720 had the lowest disease severity.

3.3.3 Resistance/tolerance to ARR

Emergence rates, root rot severity, nodulation and vigor between and within the inoculated and non-inoculated plots was significantly different ($P < 0.05$) for all the pea genotypes examined in 2016, but in 2015, none of the parameters within the inoculated and non-inoculated plots were

significant (Table 3.6). The cultivar ‘Golden’ had the highest emergence rate in the inoculated plots in 2015, while ‘Reward’ had the highest emergence in 2016. In the inoculated plots, the lowest disease severity was observed in ‘Carman’ in 2015 at both sites, while the lowest disease severity was observed in line 00-2067 in 2016 in the inoculated plots. The highest root nodulation was observed in ‘Cooper’ and ‘LN4228’ in inoculated plots in 2015 and 2016, respectively. Plant vigor in the field was greatest for ‘Carman’ in 2015, while in 2016 vigor was highest in ‘Cooper’, ‘Hornet’, ‘LN4228’ and ‘Reward’. The cultivar ‘Leroy’ had the greatest yield in both the non-inoculated plots and the inoculated plots in 2015, while ‘Saffron’ had the greatest yields in the both the non-inoculated and inoculated plots in 2016. The GGE biplot analysis indicated that line 00-2067 was the most resistant host genotype, while ‘Spring D’ had the lowest percentage yield reduction (Figs. 3.3 and 3.4).

3.4 Discussion

The results from the greenhouse tests indicated that inoculation of pea with *A. euteiches* virulence pathotype I reduced plant height, nodule numbers and vigor at the seedling stage. Root rot severity increased by more than 300% at 45 CFU mL^{-1} (the greatest increase among the 10 inoculum concentrations evaluated). This suggests that outbreaks of ARR may require a threshold level of pathogen inoculum in the soil. Cannesan et al. (2011) observed that infection by *A. euteiches* starts in the elongation zone of the pea roots, which is the region that stimulates root cell elongation. The stimulation by *A. euteiches* of border-cell production is dependent on the number of oospores. Based on the results of the inoculum density test, however, when the

number of oospores in the soil was too great, the roots could not defend against infection by *A. euteiches*. A negative linear relationship was observed between root nodulation and root rot severity, indicating that *A. euteiches* infection suppresses root nodule formation. The suppression of nodule formation also was observed for pea root rot caused by *Rhizoctonia solani* Kühn (Hwang et al. 2003). The mechanisms controlling the interactions between pea, rhizobia and *A. euteiches* remain unknown.

Under greenhouse conditions, disease severity was negatively correlated with plant height, nodulation and vigor, which again demonstrated the adverse effects of *A. euteiches* on plant health. This also shows that disease severity could be used as a parameter to determine the impact of ARR on field pea. Apron Advance + Vibrance did not prevent infection by *A. euteiches*. Seed treatment with the three other fungicides, especially Intego Solo, suppressed disease severity under controlled conditions. In contrast, under field conditions, none of the fungicides tested had a significant effect on ARR severity at the seedling stage, which may reflect complex soil conditions, the presence of other soil-borne pathogens, or the ineffectiveness of the fungicidal seed treatments. Oyarzun et al. (1990) reported that a single fungicide (fosetyl-Al) was sufficient to control ARR in the greenhouse, but not under field conditions, due to the interaction with other pathogens of the root rot complex. In a field study by Xue (2003), the biological control agent *C. rosea* strain ACM941 provided control of ARR in some years, but not others. Therefore, it appears that a single fungicide or product on its own

cannot provide acceptable control of the root rot complex in field pea. Seed treatments formulated with different fungicides and/or biological control agents likely are necessary.

In the current evaluation of resistance in field pea to *A. euteiches*, differences between inoculated and non-inoculated sites for all of the parameters were significant in 2016, but not in 2015. This may be explained by the intense rainfall that occurred in 2016, which favoured the development of ARR. All the traits measured varied significantly among the 22 pea cultivars in the field trials at Edmonton between 2015 and 2016. The cultivars 'Leroy' and 'Saffron' had the highest yields in both disease-free and inoculated plots in 2015 and 2016, respectively. Nonetheless, the GGE biplot analysis demonstrated that 'Spring D' had the lowest percentage yield reduction, so was the most stable entry for that trait. A similar analysis showed that line 00-2067 was the genotype most resistant to ARR, with the lowest disease severity and lowest increase in disease severity between inoculated and non-inoculated sites. This is consistent with the findings of Conner et al. (2013), who reported that the line 00-2067 was most tolerant to ARR in a disease nursery in Manitoba. According to Wicker et al. (2003), resistance also occurs in the pea genotypes MN313 and PI 180693 among others. Based on the performance of the 22 field pea cultivars evaluated, it seems that "ideal" cultivars which combine high yield with resistance (or tolerance) to *A. euteiches* are not available in Alberta.

The current study demonstrated that ARR adversely affects the growth of field pea at all growth stages. Seeds treated with fungicides suppressed the growth of *A. euteiches* at the seedling stage under greenhouse conditions. Fungicidal suppression of the disease under field

conditions, however, was not observed. Breeding efforts aimed at developing field pea cultivars for western Canada that have both effective ARR resistance or tolerance and good agronomic traits should be a priority, as it is likely that no single ARR management strategy will be sufficient to control this disease completely. An integrated approach to disease management, that combines partial host resistance with the use of seed treatments and the incorporation of cultural practices such as crop rotation, will be needed for the sustainable control of ARR of field pea.

3.5 References

Bødker, L., Kjøller, R., Kristensen, K., and Rosendahl, S. 2002. Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza **12:** 7-12.

Cannesan, M. A., Gangneux, C., Lanoue, A., Giron, D., Laval, K., Hawes, M. and Vicré-Gibouin, M. 2011. Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. Ann. Bot. **108:** 459-469.

Chang K. F., Hwang S. F., Ahmed, H. U., Gossen, B. D., Turnbull, G. D. and Strelkov, S. E. 2013. Management strategies to reduce losses caused by fusarium seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288.

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. 2013. Assessment of tolerance for reducing yield losses in field pea caused by Aphanomyces root rot. Can. J. Plant Sci. **93:** 473-482.

Dick, M. W. 1973. Saprolegniales. Pages 113- 144. G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman, eds. *in* The Fungi: An Advanced Treatise. Vol. IB. Academic Press, Orlando, FL.

Gaulin, E., Jacquet, C., Bottin, A. and Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol. Plant Pathol. **8**: 539-548.

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V. and Lesne, A. 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theor. Appl. Genet. **123:** 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P. and Pilet-Nayel, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biol. **13:** 1-19.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. and Persson, P. 2012. Aphanomyces pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agric. Scand.: Section B, Soil and Plant Sci. **62:** 477-487.

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D.,Howard, R. J. and Blade, S. F. 2003. Etiology, impact and control of rhizoctonia seedling blight and root rot of chickpea on the Canadian Prairies. Can. J. Plant Sci. **83:** 959-967.

Kraft, J. M. and Boge, W. L. 1996. Identification of characteristics associated with resistance to root rot caused by *Aphanomyces euteiches* in pea. Plant Dis. **80:** 1383-1386.

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D. and Turkington, T. K.

2002. Managing plant disease risk in diversified cropping systems. Agron. J. **94:** 198-209.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. Appl. Genet. **128:** 2273-2288.

Malvick, D. K. and Percich, J. A. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Dis. **83:** 51-54.

Marx, G. A., Schroeder, W. T., Provvidenti, R. and Mishanee, W. 1972. A genetic study of tolerance in pea (*Pisum sativum* L.) to Aphanomyces root rot. J. Am. Hortic. Sci. **97:** 619-621.

Oyarzun, P., Gerlagh, M., Hoogland, A. and Vos, I. 1990. Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Neth. J. Plant Pathol. **96:** 301-311.

Papavizas, G. and Ayers, W. 1974. *Aphanomyces* species and their root diseases on pea and sugarbeet. U.S. Dept. Agric. Agric. Res. Serv. Technical Bull. **1485**: 158 pp.

Pfender, W. F. and Hagedorn, D. J. 1983. Disease progress and yield loss in *Aphanomyces* root rot of peas. Phytopathology **73:** 1109-1113.

Pfender, W. F., Delwiche, P. A., Grau, C. R. and Hagedorn, D. J. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Dis. **68:** 845-847.

Pfender, W., Malvick, D. K., Pfleger, F. L. and Grau, C. R. 2001. Aphanomyces root rot. *In:* J. M. Kraft and F. L. Pfleger (eds). Compendium of pea diseases and pests. 2nd ed. APS Press, St. Paul, MN. pp. 9-13.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology **95:** 1287-1293.

R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Sauvage, H., Moussart, A., Bois, F., Tivoli, B., Barray, S. and Laval, K. 2007. Development of a molecular method to detect and quantify *Aphanomyces euteiches* in soil. FEMS Microbiol. Lett. **273:** 64-69.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., Nashville, TN, 1998 Mar. 22_25, 1998. SAS Institute, Cary, NC. pp. 1243-1246.

Scott, W. W. 1961. A monograph of the genus Aphanomyces. *In:* Technical bulletin / Virginia Agricultural Experiment Station; 151 Blacksburg, Va.: Virginia Agricultural Experiment Station, 1961.

Shehata, M. A., Davis, D. W. and Pfleger, F. L. 1983. Breeding for resistance to *Aphanomyces euteiches* root rot and *Rhizoctonia solani* stem rot in peas. J. Am. Hortic. Sci. **108:** 1080-1085.

Statistic Canada 2011. Pulses in Canada, Canadian Agriculture at a Glance, Catalogue no. 96‑325‑X, http://www.statcan.gc.ca/pub/96-325-x/2014001/article/14041-eng.htm

Thygesen, K., Larsen, J. and Bodker, L. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. Eur. J. Plant Pathol. **110:** 411-419.

Vandemark, G. J., Barker, B. M. and Gritsenko, M. A. 2002. Quantifying *Aphanomyces euteiches* in alfalfa with a fluorescent polymerase chain reaction assay. Phytopathology **92:** 265-272.

Wade, G. C. 1955. Aphanomyces root rot of peas – the effect of a potassium fertilizer on the severity of the disease in a potassium deficient soil. J. Austr. Inst. Agric. Sci. **21:** 260-263.

Wicker, E. and Rouxel, F. 2001. Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. Eur. J. Plant Pathol. **107:** 919-929.

Wicker, E., Moussart, A., Duparque, M. and Rouxel, F. 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. Eur. J. Plant Pathol. **109:** 47-60.

Wu, L. F., Chang, K. F., Fu, H., Akter, I., Li, N., Hwang, S. F. Tumbull, G. D. and Strelkov, S. E. 2017. The occurrence of and microorganisms associated with root rot of field pea in Alberta in 2016. Can. Plant Dis. Surv. **97:** 193-195.

Xue, A. G. 2003. Efficacy of *Clonostachys rosea* strain ACM941 and fungicide seed treatments for controlling the root rot complex of field pea. Can. J. Plant Sci. **83:** 519-524.

Yan, W. and Kang, M. S. 2002. GGE biplot analysis of test sites: a graphical tool for breeders, geneticists and agronomists. CRC press, Boca Raton, FL, 288 pages.

Yokosawa R., Kuninaga S. and Teranaka M. 1974. Note on pea root rot fungus, *Aphanomyces euteiches* Drechs. in Japan. Ann. Phytopathol. Soc. Japan **40:** 454-457(in Japanese).

Table 3.1. Rates of five fungicide treatments on seeds of two field pea cultivars, 'Abarth' and 'Horizon', susceptible to *Aphanomyces euteiches*.

Treatment	Fungicide	Active ingredient	Rate (mL 100 kg seeds^{-1})
Untreated control			0
Untreated control			0
Trtm 1	Apron Advance	thiabendazole (150g L^{-1}), fludioxonil (25g L^{-1}), metalaxyl (20g L^{-1})	100
	Vibrance	difenoconazole (66.2g L^{-1}), metalaxyl-M (16.5g L^{-1}), sedaxane (13.8g L^{-1})	10
Trtm 2	Intego solo	ethaboxam (383g L^{-1})	19.6
Trtm 3	BAS 516F	Boscalid (NA), pyraclostrobin (NA)	150
Trtm 4	BAS 516F	Boscalid (NA), pyraclostrobin (NA)	300
	BAS 720F	metalaxyl (NA), pyraclostrobin (NA), fluxapyroxad (NA)	100
Trtm 5	BAS 720F	metalaxyl (NA), pyraclostrobin (NA), fluxapyroxad (NA)	900

Note: NA means not available.

Table 3.2. The effect of fungicide seed treatments on a susceptible field pea cultivar, 'Horizon', grown in soil infested with *Aphanomyces euteiches* (Aphanomyces root rot) under field conditions at Edmonton, AB, in 2015-2016

Treatment	Emergence (%)		Disease severity (0 - 4)		Nodulation (0 - 4)		Vigor (0 - 4)		Yield (kg/ ha)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Non-inoc* control	81.0 a	68.5 a	0.7 c	0.6 b	0.3 bc	0.5 a	3.4 a	3.2 a	146 a	271 a
Inoc[a] control	72.6 a	65.5 a	0.9 bc	1.8 a	0.3 bc	0.7 a	2.4 b	2.7 a	145 a	206 b
Apron Advance + Vibrance	76.9 a	63.8 a	1.3 ab	2.0 a	0.6 a	0.5 a	2.7 b	2.3 a	138 a	227 ab
Intego Solo	74.3 a	66.8 a	0.9 bc	1.3 ab	0.4 ab	0.6 a	2.7 b	2.9 a	151 a	259 ab
BAS 516F	67.3 a	66.4 a	1.5 a	1.7 a	0.1 c	0.4 a	2.2 b	3.0 a	116 a	218 ab
BAS 720F+ 516F	77.4 a	64.9 a	1.6 a	1.5 ab	0.2 bc	0.7 a	2.7 b	3.1 a	137 a	220 ab
BAS 720F	80.0 a	65.6 a	1.4 a	1.4 ab	0.4 ab	0.3 a	2.7 b	3.3 a	132 a	255 ab

Note: Data are the least square mean of four replications; means in a column and category followed by the same letter do not differ based on LSD at $P < 0.05$. A macro was used with the analysis of variance to convert mean separation output to letter groupings (Saxton 1998).

*Non-inoc, non-inoculated; inoc, inoculated.

Table 3.3. The effect of fungicide seed treatments on a susceptible field pea cultivar, 'Horizon', grown in soil infested with *Aphanomyces euteiches* (Aphanomyces root rot) under field conditions at Morden, MB, in 2016

Treatment	Emergence (%)	Vigor (0 – 4)	Disease severity (0 – 4)	Nodulation (0 – 4)	Yield (kg/ ha)
Untreated	6.48 a	1.8 a	3.8 a	0 a	4.8 a
Apron Advance + Vibrance	63.0 a	2.3 a	3.7 a	0 a	5.2 a
Intego Solo	60.3 a	1.8 a	3.7 a	0 a	4.0 a
BAS 516F	63.5 a	1.8 a	3.8 a	0 a	3.6 a
BAS 720F+ 516F	65.5 a	1.5 a	3.8 a	0 a	4.4 a
BAS 720F	66.2 a	1.5 a	3.9 a	0 a	2.0 a

Note: data are the least square mean of four replications; means in a column and category followed by the same letter do not differ based on LSD at $P < 0.05$. A macro was used with the analysis of variance to convert mean separation output to letter groupings (Saxton 1998).

Table 3.4. The effect of fungicide seed treatments on two susceptible field pea cultivars, 'Abarth' and 'Horizon', inoculated with *Aphanomyces euteiches* (Aphanomyces root rot) under greenhouse conditions

Treatment	Height (mm)	Disease severity (0 - 4)	Nodulation (0 - 4)	Vigor (0 - 4)
Disease free control	115.1a	0f	3.3a	4.0a
Inoculated control	86.9de	3.6ab	1.0c	2.6c
Apron Advance + Vibrance	81.7 f	3.7a	1.0c	2.5c
Intego Solo	94.7 b	3.3de	1.3b	3.0b
BAS 516F	91.6 bc	3.4cd	1.2b	2.9b
BAS 720F+BAS 516F	83.8 ef	3.5bc	1.0c	2.6c
BAS 720F	88.2 cd	3.2e	1.2b	2.9b

Note: data are the least square mean of six replications; means in a column and category followed by the same letter do not differ based on LSD at $P < 0.05$. A macro was used with the analysis of variance to convert mean separation output to letter groupings (Saxton 1998).

Table 3.5. Pearson correlation coefficients between plant height, Aphanomyces root rot severity, root nodulation and vigor in two susceptible field pea cultivars, 'Abarth' and 'Horizon', inoculated with *Aphanomyces euteich*es under greenhouse conditions

Trait	Plant height (mm)	Disease severity (0-4)	Nodulation (0-4)
Plant height (cm)	…	…	…
Disease severity (0-4)	-0.42***[a]	…	…
Nodulation (0-4)	0.63***	-0.73***	…
Vigor (0-4)	0.55***	-0.40***	0.56***

[a]*, **, and *** indicate significant correlation at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3.6. Seedling emergence, vigor, Aphanomyces root rot severity, nodulation and yield in 22 pea pea genotypes grown *Aphanomyces euteich*es-infested field plots in Edmonton, AB, in 2015 and 2016.

Treatment	Pea genotype	Emergence (%)		Disease severity (0 – 4)		Nodulation (0 - 4)		Vigor (0 – 4)		Yield (kg/ ha)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Inoc[a]	00-2067	32.9	57.1	0.99	1.80	0.17	0.64	1.81	3.38	206	176
	Abarth	30.9	61.8	0.88	2.14	0.40	0.24	1.66	3.13	249	318
	Amarillo	10.9	37.0	1.32	2.55	0.19	0.18	0.47	2.88	101	278
	Carman	49.3	74.0	0.41	2.20	0.38	0.37	2.75	2.88	290	334
	Cooper	36.0	71.4	1.26	2.32	0.54	0.30	2.03	3.50	255	311
	Delta	37.3	69.3	0.94	2.17	0.18	0.19	1.91	3.25	188	306
	Golden	58.0	84.5	0.94	2.57	0.26	0.11	2.53	3.38	292	349
	Green water	4.0	8.2	1.32	2.32	0.29	0.28	0.09	0.87	61	72
	Horizon	34.4	63.7	1.24	2.53	0.24	0.10	1.56	3.13	211	244
	Hornet	34.9	68.7	0.93	2.53	0.29	0.11	1.81	3.50	266	335
	Leroy	45.7	75.9	1.23	2.25	0.19	0.28	2.09	2.75	337	304
	Limerick	40.6	80.2	1.07	2.15	0.41	0.31	2.00	2.88	265	299
	LN4228	24.6	76.0	1.00	2.10	0.46	0.68	1.25	3.50	201	359
	Meadow	43.6	83.9	1.44	2.24	0.21	0.14	2.06	3.25	310	349
	Patrick	34.7	73.5	1.48	2.52	0.22	0.18	1.94	3.25	279	354
	Pluto	29.0	55.0	1.01	2.56	0.31	0.06	1.56	2.75	244	236
	Raezer	40.9	77.5	0.84	2.53	0.19	0.12	2.22	3.25	245	335
	Reed bat 8	41.7	56.6	0.82	2.52	0.22	0.25	1.81	3.00	268	286
	Reward	38.7	85.0	0.90	2.34	0.30	0.33	1.84	3.50	265	278
	Saffron	44.4	83.9	1.23	2.44	0.24	0.33	2.28	3.25	301	359
	Spring D	39.6	51.3	0.95	2.30	0.39	0.36	1.56	2.75	193	287
	Striker	44.8	56.7	1.13	2.52	0.44	0.18	2.13	2.88	205	273
	LSD	2.0	3.0	0.04	0.03	0.02	0.03	0.10	0.09	10.9	11.4
	SEM	5.1	4.2	0.16	0.17	0.14	0.12	0.26	0.23	25.8	22.5
Non-inoc[a]	00-2067	31.2	67.4	0.96	1.14	0.44	0.49	1.59	3.50	238	215
	Abarth	30.3	66.1	1.15	0.48	0.19	0.69	1.78	3.88	243	351
	Amarillo	11.7	35.7	1.25	1.12	0.09	0.47	0.75	3.38	145	291
	Carman	50.3	82.8	0.60	0.42	0.23	1.03	2.56	3.13	268	357
	Cooper	36.0	67.6	1.38	1.01	0.56	0.75	2.06	3.75	275	324
	Delta	39.2	77.5	0.88	0.41	0.28	0.71	1.78	3.50	193	302

Golden	44.7	84.0	1.10	0.68	0.35	1.08	2.38	3.63	305	364
Green water	4.2	11.3	1.23	0.92	0.40	0.74	0.13	2.13	41	110
Horizon	33.0	73.8	0.99	0.81	0.20	0.48	1.38	3.50	229	299
Hornet	31.0	69.5	0.89	0.29	0.11	0.37	1.78	3.63	258	357
Leroy	43.8	82.3	1.39	0.75	0.29	0.41	2.31	2.88	334	291
Limerick	38.4	80.7	1.20	0.55	0.32	0.76	2.00	3.38	270	360
LN4228	22.6	74.1	1.07	0.65	0.44	0.84	1.44	3.63	183	355
Meadow	42.3	86.6	1.23	0.73	0.20	0.31	2.41	3.50	315	363
Patrick	32.4	76.4	1.47	0.36	0.24	0.51	1.53	3.63	276	375
Pluto	29.6	51.9	0.89	0.46	0.35	0.38	1.75	2.88	262	295
Raezer	41.0	80.0	0.98	0.54	0.30	0.35	2.44	3.38	240	340
Reed bat 8	34.4	60.9	0.81	0.32	0.27	0.96	1.72	3.38	263	312
Reward	34.1	81.2	1.04	0.75	0.30	0.86	1.78	3.63	256	336
Saffron	40.6	86.1	0.91	0.56	0.32	0.61	2.13	3.38	292	382
Spring D	36.1	57.5	1.10	0.30	0.41	0.88	1.94	3.13	222	261
Striker	44.9	60.9	1.17	0.39	0.24	0.65	2.38	3.38	228	310
LSD	1.8	3.0	0.04	0.04	0.02	0.04	0.10	0.06	10.5	10.4
SEM	5.1	3.2	0.16	0.14	0.16	0.17	0.26	0.24	31.4	17.8
P-value (inoc vs. non-inoc)	NS	0.05	NS	0.05	NS	0.05	NS	0.05	NS	0.05

[a]Non-inoc, non-inoculated; inoc, inoculated

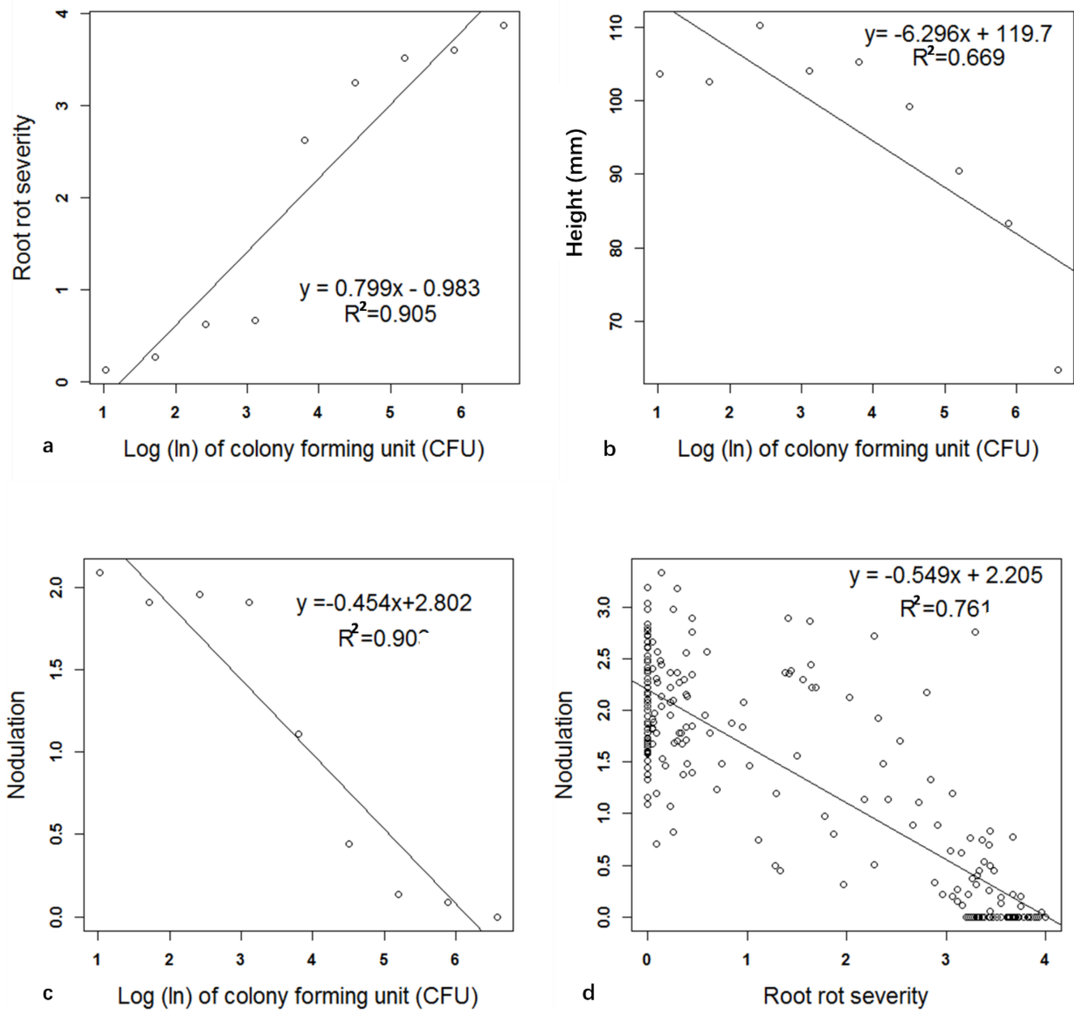

Fig. 3.1. Effect of *Aphanomyces euteiches* inoculum concentration on (a) plant height and (b) and root nodulation in field pea. The relationships between nodulation and inoculum concentration (c) and nodulation and root rot severity (d) also are shown. The graphs represent the greenhouse data obtained with the susceptible field pea cultivars ‘Abarth’ and ‘Horizon’.

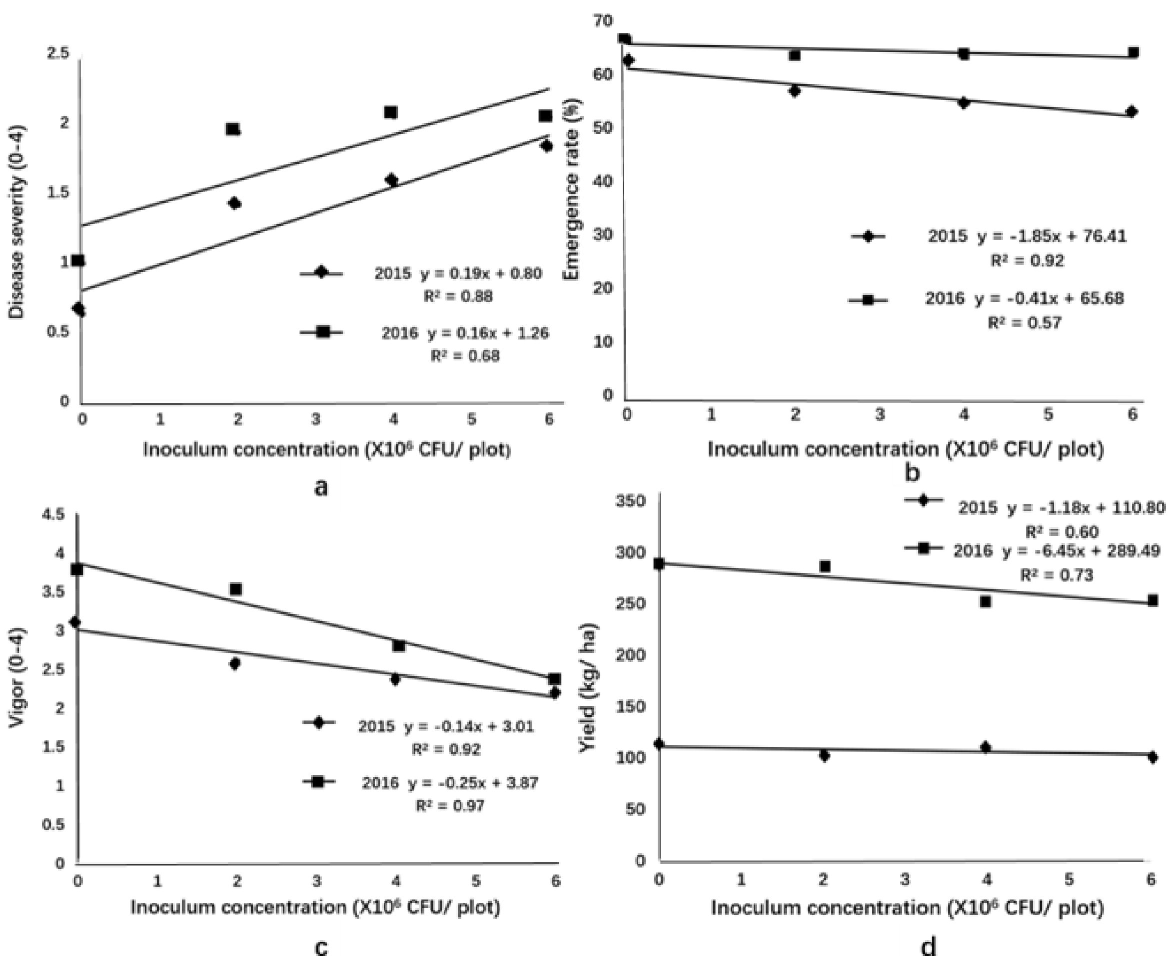

Fig. 3.2. Effect of *Aphanomyces euteiches* inoculum concentration disease severity (a), emergence rate (b) degree of wilt (vigor) (c) and yield (d) of two susceptible field pea cultivars, 'Abarth' and 'Horizon', under field conditions in Edmonton, AB, in 2015-2016.

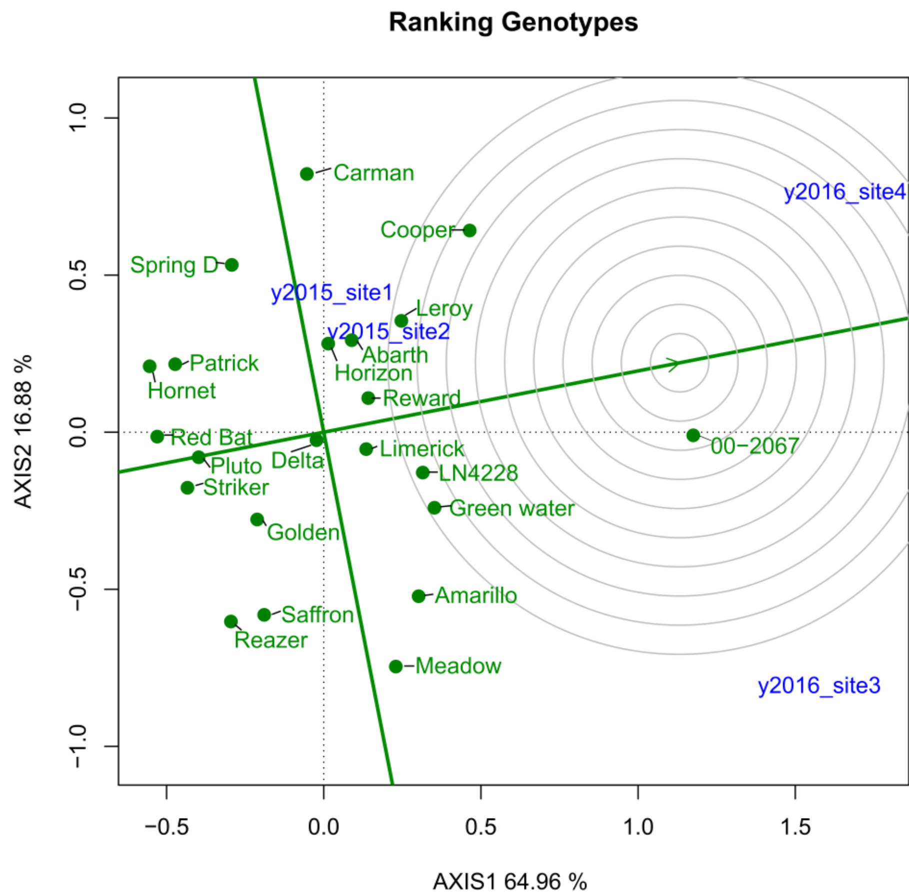

Fig. 3.3. A GGE biplot showing rankings with respect to increase in Aphanomyces root rot severity in 22 pea cultivar/lines in relation to that of a cultivar with least variance. Data were expressed as the quotient of the disease severity of the plots inoculated with *Aphanomyces euteiches* over the non-inoculated plots under field conditions in Edmonton, AB, in 2015 and 2016.

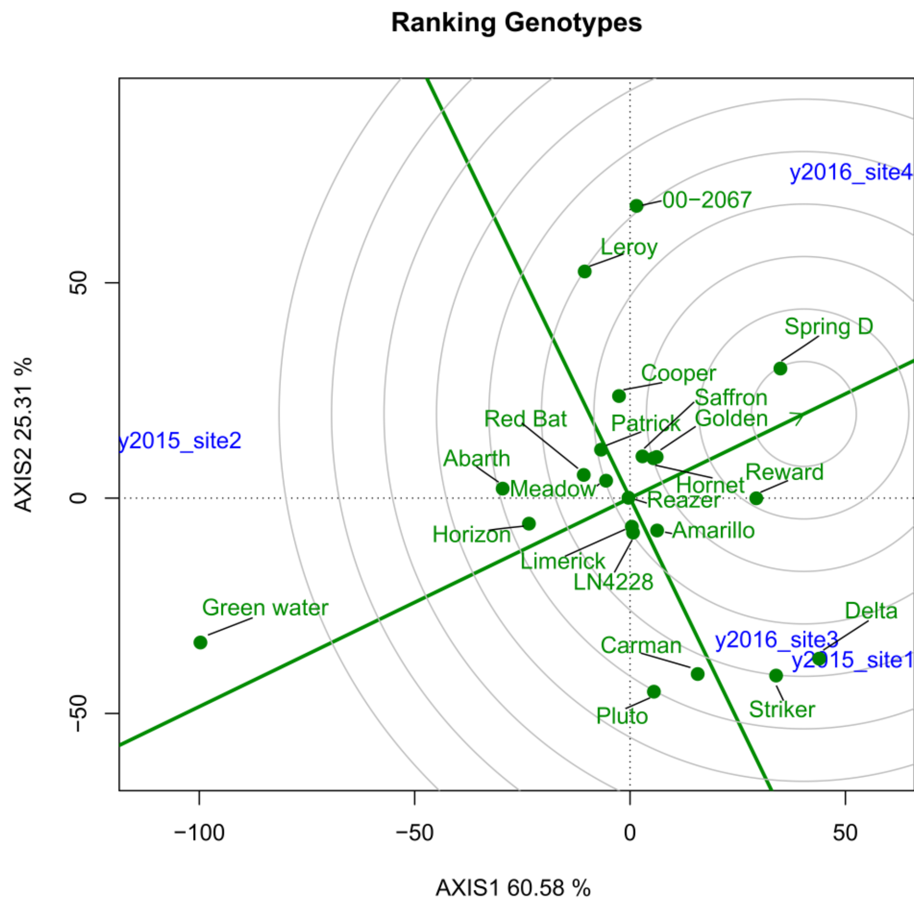

Fig. 3.4. A GGE biplot showing rankings with respect to percentage yield reduction caused by *Aphanomyces euteiches* on 22 pea cultivar/lines in relation to that of a cultivar with least variance. Data were expressed as the quotient of the yield of plots inoculated with *A. euteiches* over the non-inoculated plots under field conditions in Edmonton, AB, in 2015 and 2016.

Chapter 4: Identification of quantitative trait loci (QTL) associated with partial resistance of field pea to Aphanomyces root rot

4.1 Introduction

Field pea is an economically important, widely cultivated cool-season legume crop (Hossain et al. 2012). Pea seeds contain high protein content, are rich in carbohydrates, calcium, iron, phosphorus and various vitamins (Zhang et al. 1985; Burstin et al. 2007; Yoshida et al. 2007; Trinidad et al. 2010), and hence are a nutrient-rich food and feed for humans and animals. Canada is the largest dry pea producer in the world, with about 31% of field pea market share, followed by the European Union (30%), Russia (13%), and China (12%) (FAOSTAT 2017).

The production of field pea is affected adversely by pea root rot (Bailey et al. 2003; Xue 2003; Chang et al. 2013), which can be caused by several important soilborne pathogens including the oomycete *Aphanomyces euteiches* Drechs. (Chatterton et al. 2015). This pathogen produces oospores that can survive in the soil for up to 10 years (Papavizas and Ayers 1974; Holliday 1980). Under conducive environmental conditions, *A. euteiches* can cause yield losses as high as 86% in pea (Pfender and Hagedorn 1983). In Canada, Aphonomyces root rot (ARR) outbreaks have been reported only recently, either because pea cultivation in the same fields over multiple years under wet conditions has resulted in a build-up of the pathogen, or because the presence of *A. euteiches* can now be confirmed more easily with molecular techniques (Chatterton et al. 2015). ARR is characterized by the formation of soft and water-soaked rootlets with a honey-brown or blackish-brown color. A reduction in seedling emergence and seedling blight also has been shown to result

from ARR. As the plant grows, *A. euteiches* multiplies by the production of sporangia, causing the formation of brown lesions and cortical decay on the roots. The movement of water and nutrients in diseased plants is adversely affected, which can result in wilting and plant death (Chatterton et al. 2015).

Chemical control appears to be of limited value in the management of ARR due to a lack of effective commercial fungicides (Pilet-Nayel et al. 2002). Oyarzum et al. (1990) reported that fosetyl-Al (aluminum tris) reduced ARR severity in a seed treatment study, but only under controlled conditions. Seed treatment with hymexazol and soil drenches with azoxystrobin and propomocarb also reduced the severity of ARR on bean in Australia (Watson et al. 2013), although that study was conducted with an *A. euteiches* strain that did not infect field pea. Recently, the seed treatment product INTEGO Solo (ethaboxam; Valent, Ontario, Canada) was reported to suppress the growth of *A. euteiches* and was registered for ARR control in Canada. Several cultural disease management methods, including long rotations with non-host crops and avoiding the planting of pea in infested fields have had some success, but are not always practical (Malvick et al. 1994; Conner et al. 2013). Genetic resistance may represent the most promising way to manage ARR. Pea cultivars with complete resistance to ARR are not available, however (Pfender et al. 2001; Conner et al. 2013, Gossen et al. 2016). As a result, genotypes with only partial polygenic resistance have been used for the economic and durable control of ARR (Palloix et al. 2009; Kou and Wang 2010; Desgroux et al. 2016, Lavaud et al. 2016). Shehata et al. (1983) identified tolerance in some plant introduction (PI) lines of pea.

Partially resistant pea germplasm lines have been developed in the United States (Lockwood and Ballard 1960; Gritton 1990; Kraft 1992) and Europe (Roux-Duparque et al. 2004). Partial polygenic resistance is controlled by many quantitative trait loci (QTLs), expressing minor to major effects on the suppression of ARR (Poland et al. 2009; Kou and Wang 2010). Pyramiding and diversifying minor and major QTLs could improve the level of partial resistance or tolerance to plant diseases and reduce the frequent breakdown of major resistance genes (Castro et al. 2003; Mutlu et al. 2005; Thabuis et al. 2004; Palloix et al. 2009).

The development of marker-assisted selection (MAS) strategies offers a way to pyramid ARR resistance genes and transfer these genes into pea backgrounds with desirable agronomic traits (Pilet-Nayel et al. 2002; Hamon et al. 2011). Only a few studies have identified QTLs associated with partial resistance to *A. euteiches*. Pilet-Nayel et al. (2002, 2005) identified one major QTL *Aph1* located on the linkage group (LG) IV and five minor QTLs, which included *Aph2* on LGV, *Aph3*, *Aph4* and *Aph5* on LGI and *Aph6* on LGVII, which were associated with resistance to ARR in a population of recombinant inbred lines (RILs) of pea. The QTL *Aph1* accounted for up to 45% of the variability associated with partial resistance. Hamon et al. (2011, 2013) identified five highly stable QTLs associated with ARR resistance in pea. The QTL *Ae-Ps7.6* located on LGVII had a major effect on resistance and explained up to 56.6% of the phenotypic variation for 32 of 37 disease variables in two RIL populations. The four remaining QTLs included: *Ae-Ps1.2* on LGI, *Ae-Ps2.2* on LGII, *Ae-Ps3.1* on LGIII and *Ae-Ps4.1* located on LGIV; these accounted for up to 14.4, 26.9, 29.9 and 24.5% of the phenotypic variation,

respectively, in 13, 22, 11 or 14 of the 37 disease variables within two RIL populations. More recently, Lavaud et al. (2015) reported that the QTLs *Ae-Ps7.*6 and *Ae-Ps4.5* located on LGVII and IV, respectively, contributed significantly to ARR resistance in NILs with different genetic backgrounds. The QTL *Ae-Ps5.1* located on LGV made the smallest contribution to ARR resistance in the NILs. Thus, various studies in pea suggest that two major QTLs on LGIV and VII and several minor QTLs on LGI, II, III and V are associated with resistance to ARR in pea.

The purpose of this study was to identify simple sequence repeat (SSR) markers associated with partial resistance to ARR in a RIL pea population and evaluate the stability of the genetic loci controlling the disease reaction under disease nursery and greenhouse conditions over a two-year period.

4.2 Materials and methods

4.2.1 Plant materials

A RIL population was obtained by single-seed descent from a cross of the pea genotypes '00-2067' × 'Reward'. The ARR tolerant line '00-2067', originally developed by J.M. Kraft and V.A. Coffman (Irrigated Agriculture Research and Extension Center, Prosser, WA), has a wrinkled seed coat, white flowers and is semi-leafless (Conner et al. 2013). The susceptible cultivar 'Reward' was derived from the cross '4-0359.016' × 'MP1491' and produces white flowers and yellow cotyledons (Bing et al. 2006). The RIL population used in this study consisted of 135 individuals from the F_8 generation.

4.2.2 Isolate of *A. euteiches*

Aphanomyces euteiches isolate *Ae-MRDC1* was used in the greenhouse inoculation experiments. This isolate was recovered from soil samples collected from the ARR disease nursery at the Agriculture and Agri-Food Canada Morden Research and Development Centre, Morden (MRDC), MB (lat. 49°11' N, long. 98°5' W). The pathogen was isolated from the roots of susceptible pea plants grown as bait in *A. euteiches*-infested soil, and classified as virulence type I as described by Wicker and Rouxel (2001) (Chapter 3). The oospore inoculum was prepared in oat broth as described by Papavizas and Ayers (1974) and adjusted to a concentration of 1×10^6 oospores mL^{-1}.

4.2.3 Field trials

A two-year field experiment involving the 135 RIL lines and their parents (00-2067 and Reward) was conducted in the ARR nursery at the MRDC in 2015 and 2016. The experimental layout consisted of a generalized lattice design and was generated with CycDesigN® (VSNi, 2015). The layout differed slightly between 2015 and 2016. In 2015, each replicate consisted of 9 rows by 16 plots, with parental cultivars as checks in each row, and the two checks occurring once in each set of three rows. In 2016, the lattice layout was Latinized to account for any gradients from left to right, and up and down the field. The two replicates each with 6 rows were stacked up as a line and each row contained 24 plots consisting of 3 blocks of 8 plots; the 3 super-blocks across the field consisted of the replicates crossed by 3 blocks of 6 rows × 8 plots.

Thus, the Latinization of the lattice design created an overall array of three replicates of 3 super-blocks, each with 48 plots. The 135 RIL and three repeats of two checks occurred once in each replicate and once in each superblock. This layout was an adaptive design that could account for field gradients in both directions, which could be discerned during analysis.

The disease nursery consisted of loamy clay soil with infected pea stubble that yielded over many years very clear ARR symptoms (Conner et al. 2013). Fertilizer applications and weed control were based on standard recommendations for field pea production in the region (Saskatchewan Pulse Growers 2000). Single-row plots planted at a rate of 15 seeds per row in 2015 and 30 seeds per row in 2016 were seeded on May 7, 2015, and May 9, 2016. Emergence was determined by counting the total number of seedlings in each row on June 1, 2015, and June 8, 2016. Ten plants were dug from each plot and assessed for root rot severity on a scale of 0-9 (Conner et al. 2013), and root nodulation on a scale of 0-4 (Chang et al. 2013). Root rot rating, root nodulation and vigor were assessed in July 22, 2015, and July 19, 2016. The dry matter from the same 10 plants from each plot was separated into foliar and root portions, dried for 10 days and then weighed as a single plant average for each experimental unit. The data collected included seedling emergence (SEF) disease severity (DSF), plant vigor (VF), root weight (RWF) and foliar weight (FWF). A disease severity index (DSI) was calculated by averaging the root rot severity of each experimental unit (Papavizas and Ayers, 1974). A DSI of < 4 was regarded as indicative of tolerance, while a DSI ≥ 4 was regarded as indicative of susceptibility.

4.2.4 Greenhouse experiments

Oospore inoculum of isolate *Ae-MRDC1* was mixed to homogeneity in autoclaved sand, and then mixed again with sterilized potting mix (a granular formulation supplied by Cell-Tech, Monsanto Company, Winnipeg, MB). The inoculated potting was placed in greenhouse at room temperature (20-25°C), which was favorable for ARR development. The prepared oospore inoculum was mixed with steam-pasteurized potting mix to a concentration of 200 oospores mL^{-1} soil. Of the 135 RILs, 107 were included in the greenhouse experiments while 28 were not because of a lack of seed or because they were sister lines to those used in the field and hence likely to be genetically different at some loci. To carry out the inoculation experiments, seven seeds of the parents and each individual from the RIL population were sown in 500-mL plastic cups, which were arranged in a randomized complete block design. The inoculation experiment was repeated twice, with each experiment consisting four replicates (cups). Under greenhouse conditions, plant height was measured at the end of second week after inoculation. After 4 weeks, the pea plants were uprooted and washed. In the greenhouse experiment, the collected data included plant height (HGH), disease severity (DSGH), root weight (RWGH) and foliar weight (FWGH). Assessment of root rot severity and nodulation were carried out 28 days after seeding and inoculation on scales of 0-9 (Conner et al. 2013) scale and 0-4 (Chang et al. 2013), respectively. Root weight and foliar weight were determined after the plants were dried for one week in a dryer, set at 40°C. The DSI was calculated as above.

4.2.5 DNA extraction

Two leaf samples from each of the 107 RILs and the parents were collected from 14-day-old plants from the greenhouse experiment and stored at -80°C. A DNeasy Plant Mini Kit (Qiagen, Mississauga, ON, Canada) was used to extract the DNA from about 100 mg of leaf tissue according to the manufacturer's protocol. The DNA concentration was measured with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and adjusted to 20 ng μL^{-1} with distilled water.

4.2.6 Primer screening and RIL genotyping

A total of 212 SSR markers, reported by Loridon et al. (2005) to be well distributed along the seven linkage groups in pea, were selected and synthesized by Integrated DNA Technologies (Coralville, IA, USA). PCR assays were carried out in a 12 µL reaction mixture containing 20 ng of genomic DNA, 1× Taq buffer, 2.0 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM forward primer modified at the 5'-end with a M13 tail (Boutin-Ganache et al. 2001), 0.4 µM reverse primer, 0.2 µM fluorescently labeled M13 primer and 1.25 U *Taq* polymerase (Promega, Madison, USA). Amplifications were carried out in a Mycycler Thermal Cycler (Bio-Rad, Mississauga, ON, Canada) with 35 cycles of denaturation at 94°C for 30 s (5 min for the first cycle), annealing at a temperature that varied according to the primers used for 45 s and extension at 72°C for 1 min. An aliquot of the PCR products was separated by capillary electrophoresis on an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster city, CA). Amplified PCR products from

promising markers were separated by electrophoresis on 8% polyacrylamide gels (PAGE) at 150 V for 2 h. The size fraction of the DNA bands was revealed by silver nitrate staining, with the stained gels photographed with a UV transilluminator (Bio-Rad Canada). The molecular tests were repeated using 10% of the samples to confirm the reproducibility of the results.

Bulk segregant analysis (Michelmore et al. 1991) involving the tolerant parent, susceptible parent and three bulks each of the tolerant and susceptible RILs was conducted with each marker. Each tolerant bulk DNA comprised RIL genotypes which had the lowest root rot severity in the greenhouse and field experiments, while the susceptible bulk DNA represented susceptible RIL individuals that showed the highest disease severity. The polymorphic markers were used to genotype 107 of the 135 RILs and also for QTL analysis for the greenhouse traits. In the case of the field study, 77 RILs were genotyped and used for QTL analyses. The remaining 50 constituted sister lines from which DNA was not obtained. Thus, the linkage analysis and QTL mapping were based on 107 and 77 RILs for the greenhouse and field experiments, respectively.

4.2.7 Statistical analysis

Results for each year were subjected to analysis of variance (ANOVA) with a Latin square structure in the field trial and a RCBD structure in the greenhouse experiment. The combined cultivar means from 2015 and 2016 also were examined by ANOVA with the years as the blocking (or replicate) factor. For each analysis, the residuals were reviewed graphically for consistency with the ANOVA assumptions, and experimental unit values with residual

deviations greater than three times the standard error were set to missing. All calculations were performed with the statistical programming language, Genstat (Payne, 2016). The statistical model used for ANOVA was $\alpha_{ij} = \mu + \beta_i + \gamma_j + \varepsilon_{ij}$, where α_{ij} is the score of the *i*th RIL in the *j*th replicate, μ is the mean of all the data, β_i is the *i*th RIL effect, γ_j is the *j*th replicate effect, and ε_{ij} is the residual. The homogeneity of variances by genotype and replicate was tested using Bartlett's test. A Shapiro-Wilk test was conducted to confirm the normality of residual distributions. The least square means of the RILs were estimated from ANOVA and used for QTL analysis. Statistical differences between the lines were discerned by the LSD procedure at the 5% significance level. The parental checks were replicated three times so that differences between the checks, between the entries, and between the entries with a check had different LSDs. A further analysis was conducted using principal components analysis (PCA), in which disease severity, vigor, root and foliar weights were compared. Pearson correlation coefficients were calculated between and within the greenhouse and the field data. A Chi-squared test was conducted to determine the segregation of each polymorphic marker in the RILs for the goodness of fit to an expected allelic 1:1 ratio ($P > 0.05$).

4.2.8 QTL mapping

Genotyping data from the segregating populations were used to generate a binary data matrix. Genetic linkage analyses were carried out using the ''group'' and ''order'' commands in the software MAPMAKER/ EXP, v. 3.0b (Lander et al. 1987; Lincoln et al. 1992). A minimum

LOD score threshold of 2.5 and a recombination frequency of 0.4 were adopted. Marker order was refined using the ‘‘annealing 100 50 0.01 0.99’’ command in the CarthaGene software (De Givry et al. 2005). The Kosambi function was used to determine marker positions in centimorgans (cM). Linkage map was constructed with MapChart 2.2 (Voorrips 2002). QTL analysis was carried out using genotypic data and the phenotypic traits measured in the greenhouse and field experiments with Windows QTL Cartographer v. 2.5 (Wang et al. 2012). The association of each marker with a trait was carried out via composite interval mapping (CIM) (Zeng 1993; Jiang and Zeng 1995). The test window size was set at 10 cM and the genome was scanned at a walking speed of 2 cM. The threshold for declaring a QTL significant was determined by permutation tests using 1000 reiterations (Doerge and Churchill, 1996), and was set at $P < 0.05$. Additive × additive genetic interaction was estimated with QTLNetwork 2.1 (Yang et al. 2008).

4.3 Results

4.3.1 Disease severity under greenhouse and field conditions

In the greenhouse experiments, the pea genotype 00-2067 was tolerant to ARR with an estimated mean (± standard error, SE) DSI of 3.8 ± 1.24 and 0.9 ± 0.32. In the case of the field trials, the pooled mean DSI for 00-2067 was 6.0 ± 0.20. In contrast, ‘Reward’ was susceptible to *A. euteiches*, with a mean DSI of 7.6 ± 0.94 SE and 6.7 ± 0.97 SE in each replication under the controlled conditions and 7.9 ± 0.74 SE in the field nurseries. ANOVA of field and greenhouse

results showed a significant effect of genotype on the RIL population ($P < 0.05$). The interaction of RIL genotype × year for root rot severity in the disease nurseries was not significant, while there was a significant interaction ($P < 0.05$) of RIL genotype × site under the controlled conditions. Therefore, the field data were combined by year while the greenhouse data were separated by replication for phenotype analyses (Fig. 4.1). Based on a *t*-test, the differences in disease severity between two parental genotypes, 00-2067 and 'Reward', were significant in both the field and greenhouse experiments ($P < 0.05$).

The parental checks ranked as expected for the root rot severity caused by *A. euteiches*. Frequency distributions of the estimated means of the ARR disease severity indicated that almost all the RILs in the field trials (DS2015 and DS2016) showed a DSI of 5-9, while in the greenhouse experiments (DSGH1 and DSGH2), a continuous spread in disease severity scores from 0-9 was observed (Fig. 4.1). According to the frequency distribution, the disease severity in the field had a sharp curve centering on estimated mean DSI of 7.2 for the RIL population with a relatively low SE value (0.8), while the disease severity curves in the greenhouse were sloped more gently with larger SE values (1.8 and 2.5) (Fig. 4.1).

4.3.2 Root and foliar weights

Significant differences in root weight and foliar weight were found between the parental checks 00-2067 and 'Reward' in the field and second trial of the greenhouse experiment. The parent 00-2067 had an estimated root weight mean of 0.10 ± 0.04 g and 0.14 ± 0.03 g in the greenhouse

experiments and 0.14 ± 0.06 g in the field trials, while for ‘Reward’, the estimated means were 0.05 ± 0.02 g and 0.09 ± 0.05 g in the greenhouse and 0.08 ± 0.04 g in the disease nursery. In the case of foliar weight, the estimated means for 00-2067 in the greenhouse (5.2 ± 1.0 g and 5.7 ± 1.2 g) and field (10.8 ± 9.7 g) were larger than for ‘Reward’ in the greenhouse (2.4 ± 0.6 g and 4.2 ± 0.9 g) and field (1.9 ± 1.2 g). In addition, significant RIL genotype effects were identified in the ANOVA of root weight and foliar weight in both the field and greenhouse experiments. The root weight and foliar weight of the RIL population in both the greenhouse and field experiments had a continuous frequency distribution (Fig. 4.1). Only foliar weight in the second greenhouse experiment (FWGH2), however, had a normal distribution.

4.3.3 Plant vigor under field conditions

The tolerant parent (00-2067) looked bigger and grew better than the susceptible parent (‘Reward’) in the field experiments in 2015 (VF2015) and 2016 (VF2016). The parent 00-2067 had a significantly higher vigor score ($P < 0.05$) with an estimated pooled (2015 and 2016) mean of 3.8 ± 0.5 compared with 1.8 ± 0.6 for ‘Reward’. A significant genetic variance in the RIL population was detected in the ANOVA. The frequency distribution for vigor in the RIL population was continuous but not normally distributed (Fig. 4.1).

4.3.4 Plant height under greenhouse conditions

Differences in height between the parents (00-2067 and ‘Reward’) were significant ($P < 0.05$) in both replications of the greenhouse experiment (HGH1 and HGH2). The estimated mean plant

heights for 00-2067 were 5.1±1.6 cm and 15.7±0.9 cm in HGH1 and HGH2, respectively, while the estimated mean heights for Reward were 18.1±1.2 and 18.7±1.2 in HGH1 and HGH2, respectively. Significant RIL genotype effects were detected in the ANOVA for both replications of the greenhouse experiment. The frequency distributions of height in both replications were distributed symmetrically, centering about the estimated means of the RIL population: 18.5 cm and 18.8 cm for HGH1 and HGH2, respectively, although neither was normal (Fig. 4.1).

4.3.5 Correlations between disease severity, root weight, foliar weight, vigor and height

Pearson correlation coefficients were calculated among all the traits under field and greenhouse conditions. The Pearson correlation coefficient between DSF and DSGH2 was significant (r = 0.44, $P < 0.001$), while the correlation between DSF and DSGH1 (r = 0.14, $P > 0.05$) was not. There were no significant correlations, however, in foliar weight and root weight between pooled field and each repeat of greenhouse data. Disease severity was negatively correlated with VF, FWF and RWF (Table 4.1), In the repeated greenhouse experiments, DSGH1 was negatively correlated with HGH1, FWGH1 and RWGH1 ($P < 0.05$), while DSGH2 was positively correlated with HGH2 and negatively correlated with FWGH2 and RWGH2 ($P < 0.05$) (Table 4.2). The two repeats of all traits in the greenhouse experiment were significantly correlated with the exception of single root weight (r = 0.18) (Table 4.2).

4.3.6 Primer screening and RIL genotyping

Of the 212 SSR markers screened, 48 (22.7%) were polymorphic between the two parental genotypes 00-2067 and Reward, 137 (64.6%) were monomorphic, while 27 (12.7%) failed to amplify any product. The majority (30 of 48) of the polymorphic markers also produced clear single bands from individual RILs that resembled one of the parents and hence were used for the construction of the linkage map. Twenty six of the 30 polymoprphic markers used for screening the RILs were found on the seven linkage groups of peas: LGI (three markers), LGII (six markers), LGIII (eight markers), LGIV (one marker) LGV (two markers), LGVI (three markers) and LGVII (three markers), while four markers could not be placed on any of the linkage groups of Loridon et al. (2005).

4.3.7 Linkage map construction

At LOD score ≥ 2.5, 19 of the 30 markers were associated with five linkage groups which spanned 71.4, 54.0, 67.5, 18.1 and 21.6 cM, respectively, while the remaining 11 markers were unlinked. The markers on the first (also the largest) three linkage groups in this study were named LGI, II and III because they were coincident with the integrated linkage map of field pea reported by Loridon et al. (2005), while the last (also the smallest) two linkage groups were designated Groups IV and V because they consisted of markers which mapped to different linkage groups (Fig. 4.2). The markers on LGI, II and III identified in this study covered 45%, 23% and 24%, respectively, of the composite SSR map of pea of Loridon et al. (2005).

4.3.8 QTL mapping

4.3.8.1 Mapping of disease severity

Composite interval mapping identified one QTL, designated *PARR-LGI*, in the linkage group LGI for tolerance to ARR. *PARR-LGI* was detected at a LOD threshold of 8.8 in the second trial of the greenhouse experiment (DSGH2). Despite not being detected in the three remaining trials, *PARR-LGI* is a positive QTL for controlling partial resistance to ARR, since it explained 52.5% of the phenotypic variance (Table 4.3). At least one minor putative QTL was detected on each of LGII, LGIII and Group IV, but these did not show significance at LOD threshold ≥ 2.5 (the QTL locations detected at LOD 0.8 to 1.8). Single marker analyses showed that the SSR markers AA155 and AA160 were associated significantly ($P < 0.05$) with tolerance to ARR in three (DSF2015, DSF2016 and DSFGH2) of the four trials.

4.3.8.2 Mapping of root weight

Root weight under *A. euteiches* pressure was associated with two QTLs, designated *PRW-LGI* and *PRW-LGII*, which collectively explained 41.2 % of the phenotypic variance. The former QTL (*PRW-LGI*) is located in the same genomic region as *PARR-LGI* in the linkage group LGI but was flanked by the SSR markers AA67 and AB114. The QTL *PRW-LGI* was detected at a LOD threshold of 4.0 in the second trial of the greenhouse experiment (RWGH2), but at non-significant LOD thresholds of 1.3 and 1.9 in the field trials conducted in 2015 (RWF2015) and 2016 (RWF2016). Nonetheless, the peak position of *PRW-LGI* in RWF2015 (67.8 cM) and RWF2016 (67.2 cM) was over 40.0 cM downstream from its peak position in RWGH2 (27.0

cM). This suggests that the QTL associated with root weight on LGI could consist of two independent genomic regions. In the case of *PRW-LGII*, this QTL was flanked by the SSR markers AA238 and AB33 and was detected at a LOD threshold of 3.0 in RW2016. This QTL seems to be consistent, although two other minor QTLs detected in the same genomic region did not exceed the significance threshold. The phenotypic variance explained by *PRW-LGII* was 14% in RWGH2. Single marker analyses showed that the SSR markers AA155 (on LGI) and AA372 (on LGII) were significantly associated ($P < 0.01$) with root weight in two (RWF2016 and RWGH2) of the four trials. Another two markers, AD148 (on LGII) and AB141 (on LGIII), were significantly associated ($P < 0.05$) with root weight in RWGH1.

4.3.8.3 Mapping of foliar weight

Two genomic regions, designated *PFW-LGII-1* and *PFW-LGII-2*, in LGII was associated with foliar weight under ARR pressure. *PFW-LGII-1* was detected in the first trial of the greenhouse inoculation experiment (FWGH1) while *PFW-LGII-2* was detected in the 2015field trial (FWF2015). The two QTLs together explained 28.9% of the phenotypic variance and both were detected at LOD scores of approximately 2.5. The LOD score curves for FWF2016 and FWGH2 were similar to that for FWF2015, but these were detected at non-significant thresholds. The QTL *PFW-LGII-2* seems to be consistent since it was detected in three of the four trials. Furthermore, *PFW-LGII-2* was detected in the same genomic region as *PRW-LGII*, which suggests an association between the genetic control of foliar weight and root weight. Single

marker analyses showed that the SSR markers AB33 and AA372 were significantly associated with foliar weight in two (RWF2016 and RWGH2) of the four trials.

4.3.8.4 Mapping of plant height

One major QTL, designated *PH-LGII,* in LGII was associated with plant height in the second trial of the greenhouse experiment (HGH2) and explained 16.5% of the phenotypic variance. The LOD score profile is truncated at a maximum LOD score of 4.8. This QTL is downstream of *PFW-LGII-1*, *PFW-LGII-2* and *PRWLGII*. Single marker analysis showed that the SSR marker AA372 was significantly associated plant height in HGH2 ($P < 0.0001$).

4.3.8.5 Mapping of vigor

No significant QTLs were detected that were associated with vigor under field conditions in either 2015 (VF2015) or 2016 (VF2016).

4.4 Discussion

The parents (00-2067 and 'Reward') were significantly ($P < 0.05$) different from each other with respect to all the parameters except RWGH1, RWGH2 and FWGH2. Significant differences and variations in the RIL populations were detected for all parameters. This most likely is due to genetic differences in the parents (Conner et al. 2013), which manifested as diversity alleles in the RIL population for the traits studied. The frequency distribution of root rot severity in the 135 RILs included in the field experiment and 107 RILs in the greenhouse experiment was

continuous, but deviated from normality, which is not unusual for field disease data (Eskridge 1995; Feng et al. 2013; Coyne et al. 2015). Data transformations did not improve the distribution. This was probably due to environmental effects and the contribution of different QTLs (composed of major and minor QTLs), each of which was responsible for small increments in resistance. A bimodal trend was detected in the frequency distribution of disease severity in the greenhouse, which explained the large effect of the QTL *PDS-LGI* (77%) for tolerance to ARR. In addition, transgressive segregation was observed in the phenotype data of the RIL population for both the field and greenhouse experiments, in which some lines were more resistant and others more susceptible than the resistant and susceptible parents, respectively. Transgressive segregation of disease resistance has been reported in several other studies (Pilet-Nayel et al. 2002; Jinks and Pooni 1976; Feng et al. 2011; Li et al. 2012; Coyne et al. 2015). The factors responsible for transgressive segregation of the progeny remain unclear (Kuczyn′ ska et al. 2007), although Nakedde et al. (2016) suggested resistance genes in the parents residing on different linkage groups could account for the higher levels of tolerance exhibited by some of the RILs.

The low to moderate Pearson correlation coefficients ($r < 0.6$) detected between the field and greenhouse experiments also has been reported in several QTL mapping studies (Pilet-Nayel et al. 2002; Jung et al. 2003; Mesfin et al. 2003; Hamon et al. 2011). This perhaps might reflect the complex nature of interactions between the host and different strains of *A. euteiches* as well as other root rot microorganisms (e.g., *Fusarium*, *Phytophthora* and *Pythium* spp.). Moreover,

plants grown in the greenhouse were exposed to only a single isolate of *A. euteiches*, while those grown in the field were exposed to a larger pathogen population that may have included different virulence types. The Morden site has been used for ARR disease trials over several years, and so might have a higher inoculum density which also could have contributed to the higher root rot severity ratings observed in field vs. greenhouse conditions. Soil conditions and environmental factors (amount of precipitation, soil temperature and sunlight) also vary in the field.

Conner et al. (2013) found that ARR affects root weight, foliar weight and plant vigor in pea. In the present study, high correlations between ARR severity and these variables also were observed both in the field and greenhouse. Root weight was found to be significantly correlated with foliar weight under both field and greenhouse conditions. A similar outcome was observed by White and Castillo (1992), who also reported that 'root effects' influenced shoot dry weight in common bean under drought stress conditions. Previous studies in chickpea (Johansen et al., 1994) and snap bean (Navarra et al. 2008) reported that early growth vigor was beneficial to shoot biomass production. In this study, vigor was significantly correlated with root weight and foliar weight in the field. Therefore, these results suggest that plants which are tolerant to pathogens establish good growth of parts both above and below soil. They also suggest that, at the molecular level, the genes controlling tolerance to ARR are significant determinants of root weight, foliar weight and vigor under disease pressure. In contrast, plant height was only slightly

or not correlated with any of the measured traits, indicating that the effect of height under ARR pressure likely was distorted by other factors.

This study identified a total of six QTLs based on the traits: disease severity (*PARR-LGI*), root weight (*PRW-LGI* and *PRW-LGII*), foliar weight (*PFW-LGII-1* and *PFWLGII-2*) and plant height (*PH-LGII*). The SSR markers AA155 and AB114 found to be associated with tolerance to ARR also were reported by Hamon et al. (2011, 2013). This suggests that the partially resistant genotypes (PI 180693 and '90-2131') used to develop the RILs by Hamon et al. (2011, 2013) and the genotype 00-2067 used to develop the RILs in the present study shared some common resistance loci. However, the pedigrees of these lines are not known and so we cannot confirm this hypothesis. In this study, AA155 and AB114 mapped to linkage group (LGI), while in Hamon et al. (2011, 2013) AA155 mapped to LGI and AB114 mapped to LGVII where the major QTL *Ae-Ps7.6* is located (Hamon et al. 2011). Thus, *PARR-LGI* identified in this study and *Ae-Ps1.1* identified by Hamon et al. (2011, 2013) were coincident. *PARR-LGI* was found in the same genomic region with the QTL associated with root weight, *PRW-LGI*, and also with *Ae-Ps1.1*. The genomic region of the other QTL for root weight (*PRW-LGII*) and the two QTLs for foliar weight (*PFW-LGII-1* and *PFWLGII-2*) were coincident with the QTL *Ae-Ps2.2,* which was reported by Hamon et al. (2011) to be associated with root rot severity and aerial decline. Thus, the QTLs detected in LGI and LGII in this study as well as *Ae-Ps1.1* and *Ae-Ps2.2* reported by Hamon et al. (2011, 2013) underscored the adverse impact of ARR on pea biomass above and below ground. There is some merit in the use of root weight and shoot weight as

measures of disease severity, as was the case for resistance to Phytophthora root rot on soybean and Fusarium root rot on black bean (Lee et al. 2014; Nakedde et al. 2016).

Plant height was associated with one QTL (*PH-LGII*), which also was co-linked with *Ae-Ps2.2* (Hamon et al. 2011). Ferrari et al. (2016) reported that plant height was a useful agronomic and quality trait of field pea and under disease-free conditions identified the QTL to LGIII. Bourion et al. (2010), using the same flanking markers (AB33 and AA372) as in this study, mapped the QTL associated with plant height and nodulation to LGII. The effects of ARR on plant height are complex and hence height is not a good measure of disease severity. Although no QTL was detected with the data on vigor, this trait could still be of interest because it was used to assess QTLs associated with root rot of snap bean in a previous study (Navarro et al. 2008). Hamon et al. (2011) classified QTLs as poorly, moderately or highly stable depending on the number of traits and populations associated with the QTL as well as the years in which a study was conducted. In the present study, the two QTLs (*PARR-LGI* and *PRW-LGI*) linked to *Ae-Ps1.1*, as well as the four QTLs (*PRW-LGII*, *PFW-LGII-1*, *PFW-LGII -2* and *PH-LG2*) linked to *Ae-Ps2.2*, could be classified as moderately stable since they were identified using two to four variables.

The size of the high-density SNP pea maps ranged from 771.6 to 1389 cM (Bordat et al. 2011; Duarte et al. 2014; Sindhu et al. 2014; Coyne et al. 2015). The size of the ‘Reward’ × ‘00-2067’ linkage map in this study is much smaller (232.6 cM). As a result, the confidence intervals for the QTLs identified were very large. There is a need to fine map these genomic regions using more

efficient genotyping methods, to find more tightly linked markers for marker-assisted breeding. Future identification of the ‘Reward’ × 00-2067 genetic map using SNP markers will enable further refinement of the current genetic map. The use of a genome-wide, transcriptome-based pea single-nucleotide polymorphism (SNP) marker platform using next-generation sequencing technology also may provide a more promising way to fine map the genomic regions identified in this study. Another innovative technology, RNA-Seq-BSA, also can be applied for the rapid and efficient detection of the QTLs (Desgroux et al. 2016). More precise identification with closely linked markers will facilitate the use of the QTLs identified in this study for in pea improvement and ARR resistance breeding.

4.5 References

Bailey, K. L., Gossen, B. D., Gugel, R. K. and Morrall, R. A. A. 2003. Diseases of field crops in Canada. 3rd ed. Canadian Phytopathological Society, University Extension Press, University of Saskatchewan, Saskatoon, SK, Cananda.

Bing, D. J., Beauchesne, D., Turkington, T. K., Clayton, G., Sloan, A. G., Conner, R. L., Gan, Y., Vera, C., Gelb, D., McLaren, D., Warkentin, T. and Chang, K. F. 2006. Registration of 'Reward' field pea. Crop Sci. **46:** 2705-2706.

Bordat, A., V. Savois, M. Nicolas, J. Salse, A. Chauveau, M. Bourgeois, J. Potier, H. Houtin, C. Rond, F. Murat, P. Marget, G. Aubert and J. Burstin, 2011. Translational genomics in legumes allowed placing in silico 5460 unigenes on the pea functional map and identified candidate genes in I L. G3. Bethesda **1:** 93-103.

Boutin-Ganache, I., Raposo, M., Raymond, M. and Deschepper, C.F. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques. **31:**24-6, 28.

Bourion, V., Rizvi, S. M. H., Founier, S., Larambergue, H. D., Galmiche, F., Marget. P., Duc, G. and Burstin, J. 2010. Genetic dissection of nitrogen nutrition in pea thorough a QTL approach of root, nodule and shoot variability. Theor. Appl. Genet. **121:** 71-86.

Brun, H., Chevre, A. M., Fitt, B. D. L., Powers, S., Besnard, A. L., Ermel, M., Huteau, V., Marquer, B., Eber, F. and Renard, M. 2002. Quantitative resistance increases the

durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytol. **185:** 285-299.

Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N. and Duc, G. 2007. Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. Plant Physiol. **144:** 768-781.

Castro, A. J., Chen, X. M., Corey, A., Filichkina, T., Hayes, P. M., Mundt, C., Richardson, K., Sandoval-Islas, S. and Vivar, H. 2003. Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on adult plant resistance. Crop Sci. **43:** 2234-2239.

Chang, K. F., Hwang, S. F., Ahmed, H. U., Gossen, B. D., Turnbull, G. D. and Strelkov, S. E. 2013. Management strategies to reduce losses caused by *Fusarium* seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288-289.

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. 2013. Assessment of tolerance for reducing yield losses in field pea caused by Aphanomyces root rot. Can. J. Plant Sci.**93:** 473-482.

Coyne, C., Pilet-Nayel, M., Mcgee, R., Porter, L., Smýkal, P. and Grünwald, N. 2015. Identification of QTL controlling high levels of partial resistance to *Fusarium solani* f. sp. *pisi* in pea. Plant Breed. **134:** 446-453.

Darvasi, A. and Soller, M. 1997. A simple method to calculate resolving power and confidence interval of QTL map location. Behav. Genet. **27:** 125-132.

Desgroux, A., L'Anthoëne, V., Roux-Duparque, M., Rivière, J.-P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P., Piriou, C., McGee, R. J., Coyne, C. J., Burstin, J., Baranger, A., Manzanares-Dauleux, M., Bourion, V. and Pilet-Nayel, M. -L. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genom. 17: 124.

Doerge, R. W. and Churchill, G. A. 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285-294.

Duarte, J., N. Riviere, A. Baranger, G. Aubert, J. Burstin, L. Cornet, C. Lavaud, I. Lejeune-Henaut, J. P. Martinant, J. P. Pichon, M. L. Pilet- Nayel and G. Boutet, 2014. Transcriptome sequencing for high throughput SNP development and genetic mapping in Pea. BMC Genom. **15:** 126.

Eskridge, K. M. 1995. Statistical analysis of disease reaction data using nonparametric methods. HortSci. **30:** 478-481.

Feng, J., Hwang, R., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E. and Xue, A. G. 2011. Identification of microsatellite markers linked to quantitative trait loci controlling resistance to *Fusarium* root rot in field pea. Can. J. Plant Sci. **91:** 199-204.

Ferrari, B., Romani, M., Aubert, G., Boucherot, K., Burstin, J., Pecetti, L., Huart-Naudet, M., Klein, A. and Annicchiarico, A. 2016. Association of SNP markers with agronomic and quality traits of field pea in Italy. Czech J. Genet. Plant Breed. **52:** 83-93.

Gossen, B. D., Conner, R., Henriquez, M., Chang, K. F., Hwang, S. F., Pasche, J. and Chatterton, S. 2016. Identifying and managing root rot of pulses on the northern Great Plains. Plant Dis. **100:** 1965-1978.

Gritton, E. T. 1990. Registration of 5 root-rot resistant germplasm lines of processing pea. Crop Sci. **30:** 1166-1167.

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V. and Lesne, A. 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theor. Appl. Genet. **123:** 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P. and Pilet-Nayel, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biol. **13:** 1-19.

Holliday, P. 1980. Fungal diseases of tropical crops. Cambridge University Press, Cambridge, UK. p. 2527.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. and Persson, P. 2012. Aphanomyces pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agric. Scand.: Section B, Soil Plant Sci. **62:** 477-487.

Hwang, S. F. and Chang, K. F. 1989. Root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69:** 139-141.

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D., Howard, R. J. and Blade, S. F. 2003. Etiology, impact and control of rhizoctonia seedling blight and root rot of chickpea on the Canadian Prairies. Can. J. Plant Sci. **83:** 959-967.

Jiang. C. and Zeng. Z.B. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics **140:** 1111-1127.

Jinks, J. L. and Pooni, H. S. 1976. Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity **36:** 253-266.

Johansen, C., Krishnamurthy, L., Saxena, N.P., Sethi, S.C., 1994. Genotypic variation in moisture response of chickpea grown under line-source sprinklers in a semi-arid tropical environment. Field Crops Res. **37:** 103-112.

Jones, F. R. and Drechsler, C. 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agric. Res. **30:** 293-325.

Jung, G., Ariyarathne, H., Coyne, D. and Nienhuis, J. 2003. Mapping QTL for bacterial brown spot resistance under natural infection in field and seedling stem inoculation in growth chamber in common bean. Crop Sci. **43:** 350-357.

Kou Y. J. and Wang, S. P. 2010. Broad-spectrum and durability: understanding of quantitative disease resistance. Curr. Opin. Plant Biol. **13:** 181-185.

Kraft, J. M. 1992. Registration of 90-2079, 90-2131 and 90-2322 pea germplasms. Crop Sci. **32:** 1076-1076.

Kuczyńska, A., Surma, M. and Adamski, T. 2007. Methods to predict transgressive segregation in barley and other selfpollinated crops. J. Appl. Genet. **48:** 321-328.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, J., Lincoln, S. E. and Newsburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental populations. Genome **1:** 174-181.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. Appl. Genet. **128:** 2273-2288.

Lee, S., Rouf-Mian, M.A., Sneller, L. K., Wang, H., Dorrance, A. E. and McHale, L. K. 2014. Joint linkage QTL analyses for partial resistance to *Phytophthora sojae* in soybean using six nested inbred populations with heterogeneous conditions. Theor. Appl. Genet. **127:** 429-444.

Lockwood, J. L. and Ballard, J. C. 1960. Evaluation of pea introductions for resistance to Aphanomyces and Fusarium root rots. Michigan Quarterly Bull. **42:** 704-713.

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-Hènaut, I. and Burstin, J. 2005. Microsatellite

marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor. Appl. Genet **111:** 1022-1031.

Malvick, D. K., Percich, J. A., Pfleger, F. L., Givens, J. and Williams, J. L. 1994. Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Dis. **78:** 361-365.

McDonald, B. A. and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. Ann. Rev. Phytopathol. **40:** 349-379.

Mesfin, A., Smith, K., Dill-Macky, R., Evans, C., Waugh, R., Gustus, C. and Muehlbauer, G. 2003. Quantitative trait loci for Fusarium head blight resistance in barley detected in a two-rowed by six-rowed population. Crop Sci. **43:** 307-318.

Michelmore, R. W., Paran, I. and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88***:* 9828-9832.

Mutlu, N., Miklas, P., Reiser, J. and Coyne, D. 2005. Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). Plant Breed. **124:** 282-287.

Nakedde, T., Ibarra-Perez, F. J., Mukankusi, C., Waines, J. G. and Kelly, J. D. 2016. Mapping of QTL associated with Fusarium root rot resistance and root architecture traits in black beans. Euphytica **212:** 51-63.

Navarro, F., Sass, M. E. and Nienhuis, J. 2008. Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Sci. **48:** 962-972.

Oyarzum, P., Gerlagh, M., Hoogland, A. and Vos, I. 1990. Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Neth. J. Plant Pathol. **96:** 301-311.

Palloix, A., Ayme, V. and Moury, B. 2009. Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. New Phytol. **183:** 190-199.

Papavizas, G. and Ayers, W. 1974. *Aphanomyces* species and their root diseases on pea and sugarbeet. US Dept. Agric. Agric. Res. Serv. Tech. Bull. **1485**: 158 pp.

Parlevliet, J. E. and Zadoks, J. C. 1977. The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. Euphytica **26:** 5-21.

Pfender, W. F. and Hagedorn, D. J. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology **73:** 1109-1113.

Pfender, W., Malvick, D. K., Pfleger, F. L. and Grau, C. R. 2001. Aphanomyces root rot. *In:* J. M. Kraft and F.L. Pfleger, eds. Compendium of pea diseases and pests. 2nd ed. APS Press, St. Paul, MN. p. 913.

Pilet-Nayel, M. -L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2002. Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theor. Appl. Genet. **106:** 28-39.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology **95:** 1287-1293.

Poland, J., A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C. and Nelson, R. J. 2009. Shades of gray: the world of quantitative disease resistance. Trends Plant Sci. **14:** 21-29.

Porter, L. D. 2006. Use of phosphorous acid to manage common root rot in processing pea. Phytopathology **96:** S93-S94.

Porter, L. D. and Coffman, V. A. 2007. Impact of rolling and phosphorous acid on root rot of dry pea in the Pacific Northwest. Phytopathology **97:** S94.

Roux-Duparque, M., Boitel, C., Decaux, B., Moussart, A., Alamie, J., Pilet- Nayel, M. L. and Muel, F. 2004. Breeding peas for resistance to Aphanomyces root rot: current main outputs of three breeding programmes. *In:* Proceedings of the 5th European conference on grain legumes, Dijon, France, p. 133.

Shehata, M. A., Davis, D. W. and Pfleger, F. L. 1983. Breeding for resistance to *Aphanomyces euteiches* root rot and *Rhizoctonia solani* stem rot in peas. J. Am. Hortic. Sci. **108:** 1080-1085.

Sindhu, A., L. Ramsay, L. A. Sanderson, R. Stonehouse, R. Li, J. Condie, A. S. K. Shunmugam, Y. Liu, A. B. Jha, M. Diapari, J. Burstin, G. Aubert, B. Tar'an, K. E.

Bett, T. D. Warkentin and A. G. Sharpe, 2014. Gene-based SNP discovery and genetic mapping in pea. Theor. Appl. Genet. **127:** 2225-2241.

Thabuis, A., Palloix, A., Servin, B., Daubeze, A. M., Signoret, P., Hospital, F. and Lefebvre, V. 2004. Marker-assisted introgression of 4 *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. Mol. Breed. **14:** 9-20.

Trinidad, T. P., Mallillin, A. C., Loyola, A. S., Sagum, R. S. and Encabo, R. R. 2010. The potential health benefits of legumes as a good source of dietary fibre. Br. J. Nutr. **103:** 569-74.

Li, W. J., Feng, J., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E., Gossen B. D. and McLaren, D. L. 2012. Microsatellite DNA markers indicate quantitative trait loci controlling resistance to pea root rot caused by *Fusarium avenaceum* (Corda ex Fries) Sacc. Plant Pathol. J. **11:** 114-119.

Wang, S., Basten, C. J. and Zeng, Z.-B. 2012. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Watson, A., Browne, S. L., Snudden, M. G. and Mudford, E. M. 2013. Aphanomyces root rot of beans and control options. Australas. Plant Pathol. **42:** 321-327.

White, J. W., Castillo, J. A., 1992. Evaluation of diverse shoot genotypes on selected root genotypes of common bean under soil water deficits. Crop Sci. **32:** 762-765.

Wicker, E. and Rouxel, F. 2001. Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. Eur. J. Plant Pathol. **7:** 919-929.

Wicker, E., Hullé, M. and Rouxel, F. 2001. Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. Plant Pathol. **50:** 433-442.

Wilde, F., Schon, C. C., Korzun, V., Ebmeyer, E., Schmolke, M., Hartl, L. and Miedaner, T. 2008. Marker-based introduction of three quantitative-trait loci conferring resistance to Fusarium head blight into an independent elite winter wheat breeding population. Theor. Appl. Genet. **117:** 29-35.

Xue, A. G. 2003. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. Phytopathology **93:** 329-335.

Yang, J., Hu, C., Hu, H., Yu, R., Xia, Z., Ye, X. and Zhu, J. 2008. WTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics **24:** 721-723.

Yoshida, H., Tomiyama, Y., Saiki, M. and Mizushina, Y. 2007. Tocopherol content and fatty acid distribution of peas (*Pisum sativum* L.). J. Am. Oil Chem. Soc. **84:** 1031-1038.

Zeng, Z. B. 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972-10976.

Zhang, D., Hendricks, D. G. and Mahoney, A. W. and Cornforth, D. P. 1985.

Bioavailability of iron in green peas, spinach, bran cereal and cornmeal fed to anemic rats.

J. Food Sci. **50:** 426-428.

Table 4.1. Pearson phenotypic correlation coefficients between the different scoring criteria in field assessments of the resistance to Aphanomyces root rot (*Aphanomyces euteiches*) in a recombinant inbred line population of pea, obtained from the cross 00-2067 × 'Reward'

Trait	DSF [a]	VF	RWF
VF	-0.88***[b]		
RWF	-0.84***	0.81***	
FWF	-0.83***	0.89***	0.86***

[a] Field traits are coded as follows: DSF = pooled disease severity data, SFWF = pooled single foliar weight data, SRWF = pooled single root weight data, VF = pooled vigor data.

[b] '***' denotes a significant difference relative to 0 at $P = 0.001$.

Table 4.2. Pearson phenotypic correlation coefficients between the different scoring criteria assessed in the recombinant inbred line population of pea obtained from the cross 00-2067 × 'Reward' in greenhouse tests for resistance to Aphanomyces root rot (*Aphanomyces euteiches*)

Trait	DSGH1[a]	DSGH2	HGH1	HGH2	FWGH1	FWGH2	RWGH1
DSGH2	0.20*[b]						
HGH1	-0.36***	0.13					
HGH2	0.10	0.37***	0.40***				
FWGH1	-0.35***	0.10	0.25*	0.38***			
FWGH2	-0.19	-0.36***	0.08	0.12	0.33***		
RWGH1	-0.65***	-0.09	0.40***	-0.17	0.53***	0.20*	
RWGH2	-0.13	-0.79***	0.09	-0.36***	0.03	0.41***	0.18

[a] Greenhouse traits are coded as follows: DSGH1 = disease severity (first trial), DSGH2 = disease severity (second trial), HGH1 = plant height (first trial), HGH2 = plant height (second trial), SFWGH1 = foliar weight (first trial), SFWGH2 = foliar weight (second trial), SRWGH1 = root weight (first trial), SRWGH2 = root weight (second trial).

[b] Asterisks denote significant differences relative to 0 as follows: '***' (P = 0.001); '*' (P = 0.05), no asterisk = not significantly different.

Table 4.3. Quantitative trait loci (QTL) associated with tolerance to Aphanomyces root rot (*Aphanomyces euteiches*) in recombinant inbred lines of pea, obtained from the cross 00-2067 × 'Reward', for two field (2015 and 2016) and two greenhouse experiments (first and second trials)

QTL	Scoring criterion[a]	Linkage group[b]	Marker interval	Confidence interval (cM)[c]	Peak of putative QTL (cM)	Maximum LOD score	Additive effect[d]	R2 (%)[e]
PARR-LG1	DSGH2	LGI	AA155-AB114	36.8-53.9	45.2	8.8	3.1	52.5
PRW-LGI	RWGH2	LGI	AA67-AB114	26.2 - 71.4	27	4	2.3	34.2
PRW-LGII	RWGH2	LGII	AA238-AB33	1.5 – 37.8	27.5	3	1.3	14
PFW-LGII-1	FWGH1	LGII	AD148-AD83	0.0 -34.7	5.5	2.6	1.5	17.1
PFW-LGII-2	FWF2015	LGII	AA238-AA372	1.5 -54.0	35.8	2.5	0.9	14.3
PH-LGII	HGH2	LGII	AB33-AA372	37.8-54.0	53.8	4.8	2.1	16.5

[a] DSGH2 = disease severity in greenhouse (second trial), RWGH2 = root weight in greenhouse (second trial), FWGH1 = foliar weight in greenhouse (first trial), FWF2015 = foliar weight in field (2015), HGH2 = plant height in greenhouse (second trial).

[b] The linkage group shown in this study is coincident with that described by Loridon et al. (2005).

[c] Confidence intervals of all independent QTLs belonging to each meta-QTL, in cM Kosambi (Darvasi et al. 1997).

[d] Effect of substituting 00-2067 alleles for 'Reward' alleles at the QTL. A positive sign indicates that QTL alleles increasing resistance were contributed by the resistant parent 00-2067, whereas a negative sign indicates that the resistance alleles were contributed by the susceptible parent 'Reward'.

[e] Percentage of phenotypic variance explained by an individual QTL.

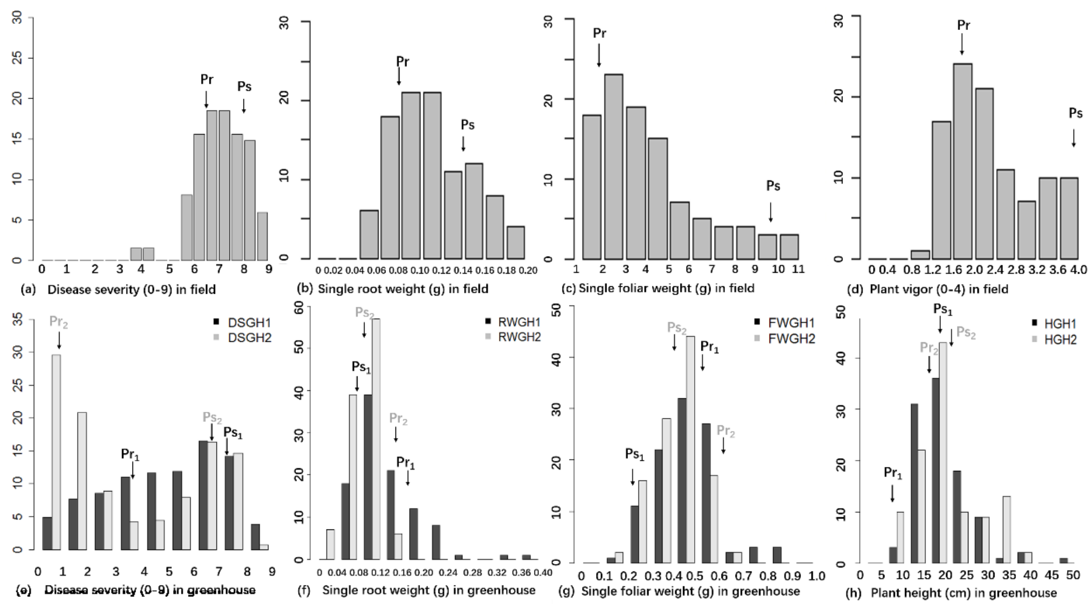

Fig. 4.1. Adjusted means frequency distributions of all variables in a recombinant inbred line population of pea, obtained from the cross 00-2067 × 'Reward', and evaluated for partial resistance to Aphanomyces root rot (*Aphanomyces euteiches*). Assessments were made under field (a-d) and greenhouse (e-h) conditions. The frequency distributions for disease severity, root weight, foliar weight and height suggested the existence of multiple genes involved in resistance to Aphanomyces root rot.

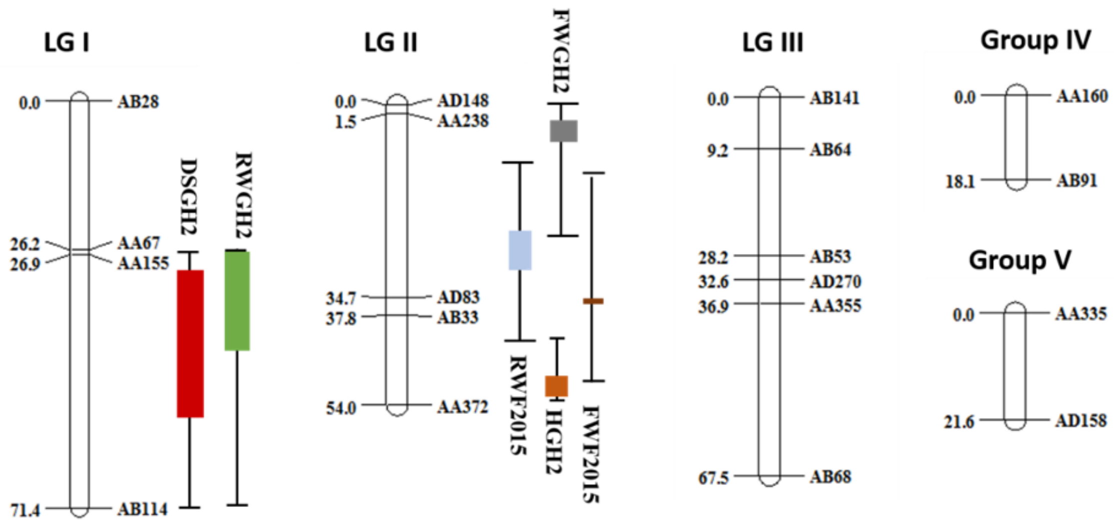

Fig. 4.2. Logarithm of odds (LOD) curve for quantitative trait loci (QTLs) for partial resistance to Aphanomyces root rot (*Aphanomyces euteiches*) identified in a recombinant inbred line population of pea, obtained from the cross 00-2067 × 'Reward', and identified under greenhouse and field conditions. The LOD profiles were obtained with Windows QTL Cartographer v. 2.5 (Wang et al. 2012).

Chapter 5: Summary and Conclusions

Aphanomyces root rot caused by *Aphanomyces euteiches* is an important disease of pea. Although it has been known as an important constraint to pea production worldwide for many decades (Pfender, 1984; Wicker et al., 2003; Gaulin et al., 2007), the management of ARR has not been studied intensely in Canada until recently (Conner et al., 2013; Chatterton et al. 2015). The work presented in this thesis was aimed at increasing understanding of the prevalence of *A. euteiches* in Alberta, evaluating effective ARR management strategies, and identifying QTLs associated with resistance to ARR in pea.

5.1 Field pea root rot survey and pathogen isolation

The incidence of and pathogens involved in causing of root rot of field pea in Alberta were examined, with a particular emphasis on evaluating the occurrence of *A. euteiches*. Root rot of field pea was more severe in 2016 than it had been in recent years. A number of microorganisms were found to be predominant among those associated with pea root rot, and included *Fusarium* spp., *Pythium* spp., *A. euteiches* and *Rizoctonia* spp. The coexistence of *A. euteiches*, *Fusarium* spp. and *Pythium* spp. in three of the six investigated areas indicated a positive correlation in the occurrence of these pathogens, which was also reported by Chatterton et al. (2015). Pure cultures of *A. euteiches* were obtained from symptomatic plants and will be characterized further in future studies for virulence phenotypes and population genetic structure. The availability of molecular technologies, such as PCR analysis and high throughput sequencing, will facilitate such studies.

5.2 Integrated management of Aphanomyces root rot of field pea

Several management strategies were evaluated for their utility in managing ARR of field pea. Linear relationships were found between inoculum concentration and disease severity; the relationships between disease severity, nodulation, seedling emergence rate, plant vigor and yield also were examined. The negative linear correlation observed between ARR severity and nodulation underscored the adverse impact of *A. euteiches* infection on the formation of root nodules on pea roots.

Five fungicidal seed treatments also were evaluated as part of this study. Compared with Apron Advance + Vibrance, which is registered on field pea but not reported to control ARR, Intego Solo was most effective at suppressing ARR under greenhouse conditions. Intego Solo is the only fungicide registered for the control of root rot caused by *A. euteiches* and *Phytophthora* spp. in Canada. In addition to Intego Solo, BAS 516F, BAS 720F and a (1:3) mix of BAS 516F and BAS 720F also helped to control ARR to different degrees in the greenhouse. Unfortunately, however, none of these treatments were effective under field conditions. The inconsistency between the field and greenhouse results may reflect the more complex environmental conditions and the presence of other root rot pathogens in the field.

Sixteen field pea cultivars registered in western Canada along with six pea lines obtained from Agriculture and Agri-Food Canada also were evaluated for ARR resistance. The pea line 00-2067 was found to be the most resistant of those evaluated in terms of ARR severity, although the cultivars 'Leroy' and 'Saffron' produced the greatest yields in 2015 and 2016, respectively. Nonetheless, line 00-2067 may serve as a useful source of ARR resistance in future pea breeding activities. It is clear that an integrated approach that includes not only

chemical control, but also genetic resistance and cultural management strategies will be required for the effective management of ARR in field pea.

5.3 Identification of QTLs associated with partial resistance or tolerance to Aphanomyces root rot in field pea

As part of this thesis project, QTLs associated with partial resistance or tolerance to ARR were identified using a RIL population derived from the cross 00-2067 × 'Reward'. Two major QTLs, *PARR-LGI* and *PRW-LGI* in LGI, explained 52.5% and 34.2% of the variation in root rot severity and root weight, respectively, in the second trial of the greenhouse experiment. Four minor QTLs, *PRW-LGII*, *PFW-LGII-1*, *PFWLGII-2* and *PH-LGII* in LGII, explained 14.0-17.1% of the variation in field and greenhouse experiments. The genomic regions containing the six identified QTLs on LGI and LGII were coincident with *Ae-Ps1.1* and *Ae-Ps2.2*, respectively, which had been reported earlier to be associated with partial resistance to ARR (Hamon et al. 2011, 2013). The eight single sequence repeat (SSR) makers flanking the six QTL could be used in pyramiding resistance to ARR in field pea.

While no completely resistant pea cultivars are available currently, partial resistance has been reported in several pea genotypes, including members of the differential set described by Wicker et al. (2001). The resistant parent, 00-2067, also represents a new ARR resistance source. Future studies should utilize additional markers, such as single nucleotide polymorphisms (SNPs) to map the QTLs identified in this study more precisely.

5.4 References

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288-289.

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. 2013. Assessment of tolerance for reducing yield losses in field pea caused by *Aphanomyces* root rot. Can. J. Plant Sci. **93:** 473-482.

Gaulin, E., Jacquet, C., Bottin, A. and Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol. Plant Pathol. **8:** 539-548.

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V. and Lesne, A. 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theor. Appl. Genet. **123:** 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P. and Pilet-Nayel, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biol. **13:** 1-19.

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D., Howard, R. J. and Blade, S. F. 2003. Etiology, impact and control of rhizoctonia seedling blight and root rot of chickpea on the Canadian Prairies. Can. J. Plant Sci. **83:** 959-967.

Jones, F. R. and Drechsler, C. 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agric. Res. **30:** 293-325.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines.Theor. Appl. Genet.**128:** 2273-2288.

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-Hènaut, I. and Burstin, J. 2005. Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor. Appl. Genet. **111:** 1022-1031.

Oyarzun, P., Gerlagh, M., Hoogland, A. and Vos, I. 1990. Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Neth. J. Plant Pathol. **96:** 301-311.

Pfender, W. F. 1984. Aphanomyces root rot. In D. J. Hagedorn (ed.), Compendium of Pea Diseases, pp. 25-28. St. Paul, MN, USA; APS Press.

Pilet-Nayel, M. -L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2002. Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theor. Appl. Genet. **106:** 28-39.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology **95:** 1287-1293.

Wicker, E. and Rouxel, F. 2001. Specific behavior of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. Eur. J. Plant Pathol. **107**: 919–929

Complete Bibliography

Abbo, S., Rachamim, E., Zehavi, Y., Zezak, I., Lev-Yadun, S. and Gopher, A. 2011. Experimental growing of wild pea in Israel and its bearing on Near Eastern plant domestication. Ann. Bot. **107:** 1399-1404.

Agriculture and Agri-Food Canada 2017. Crop Profile for Field Pea in Canada, 2015. A118-10/3-2015E-PDF

Alexopoulos, C. J. and Mims, C. W. 1979. Introductory Mycology. 3rd edition. 145-188 pp. New York, Chichester, Brisbane, Toronto.

Ali-Khan, S. T. and Zimmer, R. C. 1989. Production of field peas in Canada. Agriculture Canada. Publication 1710/E

Allmaras, R. R., Fritz, V. A., Pfleger, F. L. and Copeland, S. M. 2003. Impaired internal drainage and *Aphanomyces euteiches* root rot of pea caused by soil compaction in a fine-textured soil. Soil Tillage Res. **70:** 41-52.

Anonymous. 1996. Alberta special crop survey. 1995. Alberta Agriculture, Food and Rural Development. Market Analysis and Statistics Branch. pp. 21.

Armstrong-Cho, C., Tetreault, M., Banniza, S., Bhadauria, V. and Morrall, R. A. A. 2014. Reports of *Aphanomyces euteiches* in Saskatchewan. Can. Plant Dis. Surv. **94:** 193–194.

Badreddine, I., Lafitte, C., Heux, L., Skandalis, N. and Spanou, Z., Martinez, Y., Esquerré-Tugayé, M.T., Bulone, V., Dumas, B. and Bottin, A. 2008. Cell wall

chitosaccharides are essential components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches.* Eukaryot Cell **7:** 1980-1993.

Bailey, K. L., Gossen, B. D., Gugel, R. K. and Morrall, R. A. A. 2003. Diseases of field crops in Canada. 3rd ed. Canadian Phytopathological Society, University Extension Press, University of Saskatchewan, Saskatoon, SK, Cananda.

Bao, Y., Vuong, T., Meinhardt, C., Tiffin, P., Denny, R., Chen, S., Nguyen, H. T., Orf, J. H. and Young, N. D. 2014. Potential of association mapping and genomic selection to explore PI 88788 derived soybean cyst nematode resistance. Plant Genome **7:** 1-13. doi:10.3835/plantgenome2013.11.0039.

Basu, P. K., Brown-CreÃte, R., Gourley, C. O., Haas, J. H., Harper, F. R., Lawrence, C. H., Seaman, W. L., Toms, H. N. W., Wong, S. I. and Zimmer, R. C. 1973. Prevalence and severity of diseases of processing peas in Canada, 1970-71. Can. Plant Dis. Surv. **53:** 49-57.

Beakes, G.W., Glockling, S.L. and Sekimoto, S. 2012. The evolutionary phylogeny of the oomycete "fungi". Protoplasma **249:** 3-19.

Bekkering E. 2011. Canadian Agriculture at a Glance: Pulse in Canada. Statistics Canada. Catalogue no. 96‑325‑X, No. 007. ISSN 0-662-35659-4

Beute, M.K. and Lockwood, J.L. 1967. Pathogenic variability in *Aphanomyces euteiches*. Phytopathology **57:** 57-60.

Bing, D. J., Beauchesne, D., Turkington, T. K., Clayton, G., Sloan, A. G., Conner, R. L., Gan, Y., Vera, C., Gelb, D., McLaren, D., Warkentin, T. and Chang, K. F. 2006.

Registration of ‘Reward’ field pea. Crop Sci. **46:** 2705-2706.

Bødker, L., Kjøller, R., Kristensen, K. and Rosendahl, S. 2002. Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza **12:** 7-12.

Bordat, A., V. Savois, M. Nicolas, J. Salse, A. Chauveau, M. Bourgeois, J. Potier, H. Houtin, C. Rond, F. Murat, P. Marget, G. Aubert and J. Burstin, 2011. Translational genomics in legumes allowed placing in silico 5460 unigenes on the pea functional map and identified candidate genes in I L. G3. Bethesda **1:** 93-103.

Bourion, V., Rizvi, S. M. H., Founier, S., Larambergue, H. D., Galmiche, F., Marget. P., Duc, G. and Burstin, J. 2010. Genetic dissection of nitrogen nutrition in pea thorough a QTL approach of root, nodule and shoot variability. Theor. Appl. Genet. **121:** 71-86.

Boutin-Ganache, I., Raposo, M., Raymond, M. and Deschepper, C.F. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques. **31:**24-6, 28.

Bowers, J. H. and Parke, J. L. 1993. Epidemiology of Pythium damping-off and Aphanomyces root rot of pea after seed treatment with bacterial agents for biological control. Phytopathology **83:** 1466-1473.

Boys, C. A., Rowland, S.J., Gabor, M., Gabor, L., Marsh, I.B., Hum, S. and Callinan, R.B. 2012. Emergence of epizootic ulcerative syndrome in native fish of the Murray-

Darling River System, Australia: hosts, distribution and possible vectors. PLoS One **7:** 1-10.

Bruin, G. C. A. and Edgington, L. V. 1983. Chemical control of diseases caused by zoosporic fungi: a many-sided problem. In Zoosporic plant pathogens: A modern perspective Buczacki, S. T. (ed.) London: Academic Press. pp. 193-223.

Brun, H., Chevre, A. M., Fitt, B. D. L., Powers, S., Besnard, A. L., Ermel, M., Huteau, V., Marquer, B., Eber, F. and Renard, M. 2002. Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytol. **185:** 285-299.

Burke, D. W. and Mitchell, J. E. 1968. Temperature and moisture effects on infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology (Abstract) **58:** 1045.

Burke, D. W., Mitchell, J. E. and Hagedorn, D. J. 1969. Selective conditions for infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology **59:** 1670-1674.

Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N. and Duc, G. 2007. Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. Plant Physiol. **144:** 768-781.

Cannesan, M. A., Gangneux, C., Lanoue, A., Giron, D., Laval, K., Hawes, M. and Vicré-Gibouin, M. 2011. Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. Ann. Bot. **108:** 459-469.

Carlson, L.E. 1965. Studies on the root rot of peas caused by *Aphanomyces euteiches*. Ph.D thesis, University of Minnesota, Twin Cities, USA.

Castro, A. J., Chen, X. M., Corey, A., Filichkina, T., Hayes, P. M., Mundt, C., Richardson, K., Sandoval-Islas, S. and Vivar, H. 2003. Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on adult plant resistance. Crop Sci. **43:** 2234-2239.

Chan, M. Y. K. and Close, R. C. 1987. Aphanomyces root rot of peas: Evaluation of methods for assessing inoculum density of *Aphanomyces euteiches* in soil. New Zeal. J. Agr. Res.**30:** 213-217.

Chang, K. F., Bowness, R., Hwang, S. F., Turnbull, G. D., Howard, R. J., Lopetinsky, K., Olson, M. and Bing, D. J. 2005. Pea diseases occurring in central Alberta in 2004. Can. Plant Dis. Surv. **85:** 89-90.

Chang, K. F., Hwang, S. F., Ahmed, H. U., Gossen, B. D., Turnbull, G. D. and Strelkov, S. E. 2013. Management strategies to reduce losses caused by *Fusarium* seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chang, K., Hwang, S., Ahmed, H., Fu, H., Zhou, Q., Strelkov, S. and Turnbull, G. 2017. First report of *Phytophthora sansomeana* causing root rot in field pea in Alberta, Canada. Crop Protection **101:** 1-4. doi:10.1016/j.cropro.2017.07.008

Chang, K., Hwang, S., Ahmed, H., Gossen, B., Turnbull, G. and Strelkov, S. 2013. Management strategies to reduce losses caused by fusarium seedling blight of field pea. Can. J. Plant Sci. **93:** 619-625.

Chatterton, S., Bowness, R. and Harding, M. W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99:** 288-289.

Cheng, P., Holdsworth, W., Ma, Y., Coyne, C.J., Mazourek, M., Grusak, M.A., Fuchs, S. and McGee, R. J. 2015. Association mapping of agronomic and quality traits in USDA pea single plant collection. Mol. Breed. **35**: 75.

Chupp, C. and Sherf, A. F. 1960. Vegetable diseases and their control. New York, Ronald Press Co. 434 pp.

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. 2013. Assessment of tolerance for reducing yield losses in field pea caused by *Aphanomyces* root rot. Can. J. Plant Sci. **93:** 473-482.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M. 2000. A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genetics and Biology **30:**17-32.

Copeland, H. F. 1956. The classification of lower organisms. ix 302 pp., 45 fig. Frontis. Pacific Books, Palo Alto.

Coyne, C., Pilet-Nayel, M., Mcgee, R., Porter, L., Smýkal, P. and Grünwald, N. 2015. Identification of QTL controlling high levels of partial resistance to *Fusarium solani* f. sp. *pisi* in pea. Plant Breed. **134:** 446-453.

Daines, R. H. 1953. Capton – the new discovery in fruit fungicide. Fruit grower **73:**16.

Darvasi, A. and Soller, M. 1997. A simple method to calculate resolving power and

confidence interval of QTL map location. Behav. Genet. **27:** 125-132.

Davey, C. V. and Papavizas, G. C. 1961 Aphanomyces root rot of peas as affected by organic and mineral soil amendments. Phytopathology **51:** 131-132.

Davis, D. W., Fritz, V. A., Pfleger, F. L., Percich, J. A. and Malvick, D. K. 1995. MN 144, MN 313 and MN 314: Garden pea lines resistant to root rot caused by *Aphanomyces euteiches* Drechs. HortSci. **30:** 639-640.

Desgroux, A., L'Anthoëne, V., Roux-Duparque, M., Rivière, J., Aubert, G., Tayeh, N. and Pilet-Nayel, M. 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics 17: 124.

Dick, M. W. 1973. Saprolegniales. Pages 113- 144 G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman, eds. *in* The Fungi: An Advanced Treatise. Vol. IB. Academic Press, Orlando, FL.

Diéguez-Uribeondo, J., García, M. A., Cerenius, L., Kozubíková, E., Ballesteros, I., Windels, C., Weiland, J., Kator, H., Söderhäll, K. and Martín, M.P. 2009. Phylogenetic relationships among plant and animal parasites, and saprotrophs in Aphanomyces (Oomycetes). Fungal Genet. Biol. **46:** 365-376.

Doerge, R. W. and Churchill, G. A. 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285-294.

Duarte, J., N. Riviere, A. Baranger, G. Aubert, J. Burstin, L. Cornet, C. Lavaud, I. Lejeune-Henaut, J. P. Martinant, J. P. Pichon, M. L. Pilet- Nayel and G. Boutet,

2014. Transcriptome sequencing for high throughput SNP development and genetic mapping in Pea. BMC Genom. **15:** 126.

Eskridge, K. M. 1995. Statistical analysis of disease reaction data using nonparametric methods. HortSci. **30:** 478-481.

FAOSTAT 2017. Food and Agriculture Organization of the United Nations Statistics. http://www.fao.org/faostat/en/#data/QC

Feng, J., Hwang, R., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E. and Xue, A. G. 2011. Identification of microsatellite markers linked to quantitative trait loci controlling resistance to Fusarium root rot in field pea. Can. J. Plant Sci. **91:** 199-204.

Feng, J., Hwang, R., Chang, K. F., Hwang, S. F., Strelkov, S. E., Gossen, B. D., Conner, R. L. and Turnbull, G. D. 2010. Genetic variation in *Fusarium avenaceum* causing root rot on field pea. Plant Pathol. **59:** 413-421.

Ferrari, B., Romani, M., Aubert, G., Boucherot, K., Burstin, J., Pecetti, L., Huart-Naudet, M., Klein, A. and Annicchiarico, A. 2016. Association of SNP markers with agronomic and quality traits of field pea in Italy. Czech J. Genet. Plant Breed. **52:** 83-93.

Filipová, L., Petrusek, A., Matasová, K., Delaunay, C. and Grandjean, F. 2013. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in populations of the signal crayfish *Pacifastacus leniusculus* in France: Evaluating the threat to native crayfish. Plos One **8:** 1-10.

Fritz, V. A., Allmaras, R., Pfleger, F. L. and Davis, D. W. 1995. Oat residue and soil compaction influences on common root rot (*Aphanomyces euteiches*) of peas in a fine-textured soil. Plant Soil **171:** 235-244.

Garrett, S. D. 1944. Root disease fungi: A treatise on the epidemiology of soil-borne disease in crop plants, and a first exposition of the principles of root disease control. Massachusetts: Chronic a Botanica p. 177.

Gaulin, E., Jacquet, C., Bottin, A. and Dumas, B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol. Plant Path. **8:** 539-548.

Gaulin, E., Madoui, M.A., Bottin, A., Jacquet, C., Mathé, C., Couloux, A., Wincker, P. and Dumas, B. 2008. Transcriptome of *Aphanomyces euteiches*: new oomycete putative pathogenicity factors and metabolic pathways. PloS One **3:** e1723.

Gaumann, E. A. 1952. The fungi. (Trans. By F.L. Wynd.) 420 pp., 440 figs. Hafner Publishing Co., New York.

Gaumann, E. A. 1963. Die Pilze. 541pp., 610 figs. Birkhaouser Verlag, Basel.

Gossen, B. D., Conner, R. L., Henriquez, M., Chang, K. F., Hwang, S. F., Pasche, J. and Chatterton, S. 2016. Identifying and managing root rot of pulses on the northern Great Plains. Plant Dis. **100:** 1965-1978.

Gritton, E. T. 1990. Registration of 5 root-rot resistant germplasm lines of processing pea. Crop Sci. **30:** 1166-1167.

Gritton, E. T., Parke, J. L., Percich, J. A., Fritsz,. Y. A., Kraft, J. M., Grau, C. R, Pfleger, F. L. and Allmaras, R. R. 1995. Integrated control of Aphanomyces root rot of pea (*Pisum sativum* L.). 2nd European Conference of Grain Legumes: Improving production and utilisation of grain legumes. pp. 148-149.

Grünwald N. J. 2003. The biology of the genus Aphanomyces. Proceedings of the 2nd Inaternational Aphanomyces Workshop. United States Department of Agriculture. 9-14 pp.

Guerriero, G., Avino, M., Zhou, Q., Fugelstad, J., Clergeot, P.-H. and Bulone, V. 2010. Chitin synthases from Saprolegnia are involved in tip growth and represent a potential target for antioomycete drugs. PLoS Pathog. **6:** e1001070.

Haenseler, C. M. 1926. Studies on the root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches* Drechsler. New Jersey Agricultural Experiment Station 46th Annual Report **46:** 467-484.

Hagedorn, D. J. 1984. Compendium of pea diseases / edited by Donald J. Hagedorn. *In:* Disease compendium series St. Paul, Minnisoda: American Phytopathological Society in cooperation with Deptartment of Plant Pathology, University of Wisconsin, Madison, 1984.

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V. and Lesne, A. 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theor. Appl. Genet.**123:** 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P. and Pilet-Nayel, M. 2013. QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant Biol. **13:** 1-19.

Henig, R. M. 2000. The monk in the garden: how Gregor Mendel and his pea plants solved the mystery of inheritance. New York: Houghton Mifflin 2000.

Heyman, F., Lindahl, B., Persson, L., Wikstrom, M. and Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea. Soil Biol. Biochem. **39:** 2222-2229.

Hoch, H. C. and Mitchell, J. E. 1973. The effects of osmotic water potentials on *Aphanomyces euteiches* during zoosporogenesis. Can. J. Bot. **51:** 413-420.

Holliday, P. 1980. Fungal diseases of tropical crops. Cambridge University Press, Cambridge, UK. p. 2527.

Hossain, S., Bergkvist, G., Berglund, K., Glinwood, R., Kabouw, P., Mårtensson, A. and Persson, P. 2014. Concentration- and time-dependent effects of isothiocyanates produced from Brassicaceae shoot tissues on the pea root rot pathogen *Aphanomyces euteiches*. J. Agric. Food Chem. **62:** 4584-4591.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. and Persson, P. 2012. Aphanomyces pea root rot disease and control with special reference to impact of

Brassicaceae cover crops. Acta Agric. Scand.: Section B, Soil and Plant Sci. **62:** 477-487.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. and Persson, P. 2012. Aphanomyces pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agric. Scand.: Section B, Soil Plant Sci. **62:** 477-487.

Hwang, S. F. and Chang, K. F. 1989. Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69:** 139-141.

Hwang, S. F., Gossen, B. D., Chang, K. F., Turnbull, G. D., Howard, R. J. and Blade, S. F. 2003. Etiology, impact and control of rhizoctonia seedling blight and root rot of chickpea on the Canadian Prairies. Can. J. Plant Sci. **83:** 959-967.

Hwang, S. F., Lopetinsky, K. and Evans, I. R. 1991. Effects of seed infection by *Ascochyta* spp., fungicide seed treatment, and cultivar on yield parameters of field pea under field conditions. Can. Plant Dis. Surv.**71:** 169-172.

Hwang, S.F. and Chang, K.F. 1989. Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69:** 139-141.

Jean L., W., F.L., P., Vincent A., F. and R.R., A. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Plant and soil **188:** 43–48.

Jermyn, W. A., Banfield, R. A., Hedley, J. and Russell, A. C. 1982. Effect of seed treatment on Aphanomyces root rot of peas. Proceedings of the Agronomy Society of New Zealand **12:** 15-18.

Jiang, C. and Zeng, Z.B. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics **140:** 1111-1127.

Jinks, J. L. and Pooni, H. S. 1976. Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity **36:** 253-266.

Johansen, C., Krishnamurthy, L., Saxena, N.P., Sethi, S.C., 1994. Genotypic variation in moisture response of chickpea grown under line-source sprinklers in a semi-arid tropical environment. Field Crops Res. **37:** 103-112.

Jones, F. R. and Drechsler, C. 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agr. Res. **30:** 293-325.

Jung, G., Ariyarathne, H., Coyne, D. and Nienhuis, J. 2003. Mapping QTL for bacterial brown spot resistance under natural infection in field and seedling stem inoculation in growth chamber in common bean. Crop Sci. **43:** 350-357.

Karin, T., John, L. and Lars, B. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. Eur. J. Plant Path. **110:** 411-419.

Katan, J. 1987. Soil solarization. Pages 77-105 *In:* Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York. 372 pp.

Kerr, A. 1964. Influence of soil moisture on infection of peas by *Pythium ultimum.* Aust. J. Biol. Sci. **17:** 676-685.

King, E. B. and Parke, J. L. 1993. Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis. **77:** 1185-1188.

King, T. H. and Bissonnette, H. L. 1954. Physiologic specialization in *Aphanomyces euteiches* (Abstract). Phytopathology **44:** 495.

Knott, C. M. 1987. A key for stages of development of the pea (*Pisum sativum*). Ann. Appl. Biol. **111:** 233-245.

Kotova, V. V. and Tsvetkova, N. A. 1980. Effectiveness of chemical control measures against Aphanomyces root rot of peas. Rev. Plant Pathol. (Abstract) **59:** 342.

Kou, Y. J. and Wang, S. P. 2010. Broad-spectrum and durability: understanding of quantitative disease resistance. Curr. Opin. Plant Biol. **13:** 181-185.

Kraft, J. M. 1992. Registration of 90-2079, 90-2131 and 90-2322 pea germplasms. Crop Sci. **32:** 1076-1076.

Kraft, J. M. and Boge, W. L. 1996. Identification of characteristics associated with resistance to root rot caused by *Aphanomyces euteiches* in pea. Plant Dis. **80:** 1383-1386.

Kraft, J. M. and Kaiser, W. J. 1993. Breeding for Stress Tolerance in Cool-Season Food Legumes. *In:* K. B. Singh and M. C. Saxena (eds.). Chichester, UK: John Wiley and Sons. pp. 123-144.

Kraft, J. M., Marcinkowsha, J. and Muehlbauer, F. J. 1990. Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet-sieving/baiting technique. Plant Dis. **74:** 716-718

Kroon, L.P.N.M., Brouwer, H., de Cock, A.W. A M. and Govers, F. 2012. The genus *Phytophthora anno* 2012. Phytopathology **102:** 348-364.

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D. and Turkington, T. K., 2002. Managing plant disease risk in diversified cropping systems. Agron. J. **94:** 198-209.

Kuczyńska, A., Surma, M. and Adamski, T. 2007. Methods to predict transgressive segregation in barley and other selfpollinated crops. J. Appl. Genet. **48:** 321-328.

Laflamme, P. 1998. Diseases of field pea, *Pisum sativum* L., in the Peace River Region of Alberta. M.Sc. Thesis

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, J., Lincoln, S. E. and Newsburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental populations. Genome **1:** 174-181.

Lavaud, C., Baviere, M., Le Roy, G., Hervé, M. R., Moussart, A., Delourme, R. and Pilet-Nayel, M. 2016. Single and multiple resistance QTL delay symptom appearance and slow down root colonization by *Aphanomyces euteiches* in pea near isogenic lines. BMC Plant Biol. 16: 166.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. App. Genet. **128:** 2273-2288.

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A. and Pilet-Nayel, M. 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. Appl. Genet. **128:** 2273-2288.

Lawson, H. M. and Topham, P. B. 1985. Competition between annual weeds and vining peas grown at a range of population densities: Effects on the weeds. Weed Res. **25:** 221-229.

Leclerc, M. C., Guillot, J. and Deville, M. 2000. Taxonomic and phylogenetic analysis of Saprolegniaceae (Oomycetes) inferred from LSU rDNA and ITS sequence comparisons. Antonie van Leeuwenhoek **77:** 369-377.

Lee, S., Rouf-Mian, M.A., Sneller, L. K., Wang, H., Dorrance, A. E. and McHale, L. K. 2014. Joint linkage QTL analyses for partial resistance to *Phytophthora sojae* in soybean using six nested inbred populations with heterogeneous conditions. Theor. Appl. Genet. **127:** 429-444.

Leipe, D. D., Wainright, P. O., Gunderson, J. H., Porter, D., Patterson, D. J., Valois, F., Himmerich, S. and Sogin M. L. 1994. The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. Phycologia **33:** 369-377.

Li, W. J., Feng, J., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E., Gossen B.

D. and McLaren, D. L. 2012. Microsatellite DNA markers indicate quantitative trait loci controlling resistance to pea root rot caused by *Fusarium avenaceum* (Corda ex Fries) Sacc. Plant Pathol. J. **11:** 114-119.

Lockwood, J. L. and Ballard, J. C. 1960. Evaluation of pea introductions for resistance to Aphanomyces and Fusarium root rots. Michigan Quarterly Bull. **42:** 704-713.

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-He`naut, I. and Burstin, J. 2005. Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor. App. Genet. **111:** 1022-1031.

Malvick, D. K. and Grau, C. R. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Dis. **85:** 740-744.

Malvick, D. K. and Percich, J. A. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Dis. **83:** 51-54.

Malvick, D. K., Percich, J. A., Pfleger, F. L., Givens, J. and Williams, J. L. 1994. Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Dis. **78:** 361-365.

Malvick, D.K. and Percich, J.A. 1998a. Genotypic and pathogenic diversity among pea-infecting strains of *Aphanomyces euteiches* from Central and Western United States. Phytopathology **88:** 915-921.

Malvick, D.k. and Percich, J.A. 1998b. Variation in pathogenicity and genotype among single-zoospore strains of *Aphanomyces euteiches*. Phytopathology **88:** 52-57

Malvick, D.K. and Percich, J.A. 1999. Identification of *Pisum sativum* germplasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. Plant Dis. **83:** 51–54.

Manning, M. and Menzies, S.A. 1984. Pathogenic variability in isolates of *Aphanomyces euteiches* from New Zealand soils. New Zeal. J. Agr. Res.**27:** 569-574.

Marx, G. A., Schroeder, W. T., Provvidenti, R. and Mishance, W. 1972. A genetic study of tolerance in pea (*Pisum sativum* L.) to Aphanomyces root rot. J. Am. Soc. Hortic. Sci. **97:** 619-621.

Marx, G. A., Schroeder, W. T., Provvidenti, R. and Mishanee, W. 1972. A genetic study of tolerance in pea (*Pisum sativum* L.) to Aphanomyces root rot. J. Am. Hortic. Sci. **97:** 619-621.

Matari, N.H. and Blair, J.E. 2014. A multilocus timescale for oomycete evolution estimated under three distinct molecular clock models. BMC Evol. Biol. **14:** 1-21.

McDonald, B. A. and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. Ann. Rev. Phytopathol. **40:** 349-379.

Meisrimler, C., Wienkoop, S., Lyon, D., Geilfus, C. and Lüthje, S. 2016. Long-term iron deficiency: Tracing changes in the proteome of different pea (*Pisum sativum* L.) cultivars. J. Proteomics **140:** 13-23. doi:10.1016/j.jprot.2016.03.024

Mélida, H., Sandoval-Sierra, J. V, Diéguez-Uribeondo, J. and Bulone, V. 2013. Analyses of extracellular carbohydrates in oomycetes unveil the existence of three different cell wall types. Eukaryote Cell **12:** 194-203.

Mesfin, A., Smith, K., Dill-Macky, R., Evans, C., Waugh, R., Gustus, C. and Muehlbauer, G. 2003. Quantitative trait loci for Fusarium head blight resistance in barley detected in a two-rowed by six-rowed population. Crop Sci. **43:** 307-318.

Michelmore, R. W., Paran, I. and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88***:* 9828-9832.

Mitchell, J. E. and Yang, C. Y. 1966. Factors affecting growth and development of *Aphanomyces euteiches*. Phytopathology **56:** 917-922.

Moussart, A., Onfroy, C., Lesne, A., Esquibet, M., Grenier, E. and Tivoli, B. 2007. Host status and reaction of *Medicago truncatula* accessions to infection by three major pathogens of pea (*Pisum sativum*) and alfalfa (*Medicago sativa*). Eur. J. Plant Pathol. **117:** 57-69.

Muehlchen, A M., Rand, R. E. and Parke, J. L. 1990. Evaluation of crucifer green manures for controlling Aphanomyces root rot of peas. Plant Dis. **74:** 651-654.

Mutlu, N., Miklas, P., Reiser, J. and Coyne, D. 2005. Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). Plant Breed. **124:** 282-287.

Nakedde, T., Ibarra-Perez, F. J., Mukankusi, C., Waines, J. G. and Kelly, J. D. 2016. Mapping of QTL associated with Fusarium root rot resistance and root architecture traits in black beans. Euphytica **212:** 51-63.

Nars, A., Lafitte, C., Chabaud, M., Drouillard, S., Mélida, H., Danoun, S. and Bottin, A. 2013. *Aphanomyces euteiches* cell wall fractions containing novel glucan chitosaccharides induce defense genes and nuclear calcium oscillations in the plant host *Medicago truncatula*. PLoS One **8:** 1-13.

Navarro, F., Sass, M. E. and Nienhuis, J. 2008. Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Sci. **48:** 962-972

Olofsson, J. 1967. Root rot of canning and freezing peas in Sweden. Acta Agr. Scand. **17:** 101-107.

Ondrej, M., Dostalova, R. and Trojan, R. 2008. Evaluation of virulence of *Fusarium solani* isolates on pea. Plant Prot. Sci. **44:** 9-18.

Oyarzun, P., Dijst, G., Zoon, F. and Maas, P. 1997. Comparison of soil receptivity to *Thielaviopsis basicola*, *Aphanomyces euteiches*, and *Fusarium solani* f. sp. *pisi* causing root rot in pea. Phytopathology **87:** 534-541.

Oyarzun, P., Gerlagh, M., Hoogland, A. and Vos, I. 1990. Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Nether. J. Pathol. **96:** 301-311.

Palloix, A., Ayme, V. and Moury, B. 2009. Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. New Phytol. **183:** 190-199.

Papavizas, G. and Ayers, W. 1974. *Aphanomyces* species and their root diseases on pea and sugarbeet. U.S. Dept. Agric. Agric. Res. Serv. Technical Bull. **1485**: 158 pp.

Papavizas, G. C. 1966. Suppression of Aphanomyces root rot of peas by cruciferous soil amendments. Phytopathology **56:** 1071-1075.

Papavizas, G. C. 1967. Comparison of treatments suggested for control of Aphanomyces root rot of peas. Plant Dis. Rep. **51:** 125-129.

Papavizas, G. C. and Lewis, J. A. 1971. Effect of amendments and fungicides on Aphanomyces root rot on peas. Phytopathology **61:** 215-220.

Parke, J. L., Rand, R. E., Joy, A. B. and King, E. B. 1991. Biological control of Pythium damping-off and Aphanomyces root rot of peas by application of *Pseudomonas cepacia* or *P. fluorescens* to seed. Plant Dis. 75: 987-992.

Parlevliet, J. E. and Zadoks, J. C. 1977. The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. Euphytica **26:** 5-21.

Pfender, W. F. 1984. Aphanomyces root rot. In D. J. Hagedorn (ed.), Compendium of Pea Diseases, St. Paul, MN, USA: APS Press. pp. 25-28.

Pfender, W. F., Delwiche, P. A., Grau, C. R. and Hagedorn, D. J. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Dis. **68:** 845-847.

Pfender, W. F. and Hagedorn, D. J. 1983. Disease progress and yield loss in *Aphanomyces* root rot of peas. Phytopathology **73**: 1109-1113.

Pfender, W., Malvick, D. K., Pfleger, F. L. and Grau, C. R. 2001. Aphanomyces root rot. Pages 9-13. *In:* J. M. Kraft and F. L. Pfleger (eds.) Compendium of pea diseases and pests. 2nd ed. APS Press, St. Paul, MN.

Pilet-Nayel M. L., Coyne C., Hamon C., Lesne´ A., Le Goff I., Esnault R., Lecointe R., Roux-Duparque M., McGee R., Mangin P., McPhee K., Moussart A. and Baranger A. 2007. Understanding genetics of partial resistance to Aphanomyces root rot in pea for new breeding prospects. *In:* Third International Aphanomyces workshop on legumes, 07–09 November 2007, Rennes, France, p. 36.

Pilet-Nayel, M. -L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2002. Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. Theor. Appl. Genet. **106:** 28-39.

Pilet-Nayel, M. L., Muehlbauer, F. J., McGee, R. J., Kraft, J. M., Baranger, A. and Coyne, C. J. 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. Phytopathology **95:** 1287-1293.

Poland, J., A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C. and Nelson, R. J. 2009. Shades of gray: The world of quantitative disease resistance. Trends Plant Sci. **14:** 21-29.

Porter, L. D. 2006. Use of phosphorous acid to manage common root rot in processing pea. Phytopathology **96:** S93-S94.

Porter, L. D. and Coffman, V. A. 2007. Impact of rolling and phosphorous acid on root rot of dry pea in the Pacific Northwest. Phytopathology **97:** S94.

R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Reiling, T. P., King, T. H. and Fields, R. W. 1960. Soil indexing for pea root rot and the effect of root rot on yield. Phytopathology **50:** 287-290.

Roux-Duparque, M., Boitel, C., Decaux, B., Moussart, A., Alamie, J., Pilet- Nayel, M. L. and Muel, F. 2004. Breeding peas for resistance to Aphanomyces root rot: current main outputs of three breeding programmes. *In:* Proceedings of the 5th European conference on grain legumes, Dijon, France, p. 133.

Royo, F. andersson, G., Bangyeekhun, E., Múzquiz, J. L., Söderhäll, K. and Cerenius, L. 2004. Physiological and genetic characterisation of some new *Aphanomyces* strains isolated from freshwater crayfish. Vet. Microbiol. **104:** 103-112.

Sauvage, H., Moussart, A., Bois, F., Tivoli, B., Barray, S. and Laval K. 2007. Development of a molecular method to detect and quantify *Aphanomyces euteiches* in soil. FEMS Microbiol. Lett. **273:** 64-69.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., Nashville, TN, 1998 Mar. 22_25, 1998. SAS Institute, Cary, NC. pp. 1243-1246.

Scharen, A. L. 1960. Germination of oospores of *Aphanomyces euteiches* embedded in plant debris. Phytopathology **50:** 274-277.

Schrum, H., Kotcon, J. and Verlinden, S. 2008. Organic Methods for Control of Root Rot in Pea and Spinach in Northeastern U.S., 16th IFOAM Organic World Congress, Modena, Italy, June 16-20, 2008. pp. 624-627.

Scott, W. W. 1961. A monograph of the genus Aphanomyces. *In:* Technical bulletin / Virginia Agricultural Experiment Station; 151 Blacksburg, Va.: Virginia Agricultural Experiment Station, 1961.

Sekizaki, H., Yokosawa, R., Chinen, C., Adachi, H. and Yamane, Y. 1993. Studies on zoospore attracting activity: Synthesis of isoflavones and their attracting activity to *Aphanomyces euteiches* zoospore. Biol. Pharm. Bull. **16:** 698-701.

Shaffer, R. L. 1975. The major groups of Basidiomycetes. Mycologia **67:** 1-18.

Shehata, M. A., Davis, D. W. and Pfleger, F. L. 1983. Breeding for resistance to *Aphanomyces euteiches* root rot and *Rhizoctonia solani* stem rot in peas. J. Am. Hortic. Sci. **108:** 1080-1085.

Sherwood, R. T. and Hagedorn, D. J. 1958. Determining the common root rot potential of pea fields. *In:* Bulletin / University of Wisconsin. Agricultural Experiment Station; 531 Madison: Agricultural Experiment Station, University of Wisconsin, 1958.

Sherwood, R. T. and Hagedorn, D. J. 1962. Studies on the biology of *Aphanomyces euteiches*. Phytopathology **52:** 150-154.

Sindhu, A., L. Ramsay, L. A. Sanderson, R. Stonehouse, R. Li, J. Condie, A. S. K. Shunmugam, Y. Liu, A. B. Jha, M. Diapari, J. Burstin, G. Aubert, B. Tar'an, K. E. Bett, T. D. Warkentin and A. G. Sharpe, 2014. Gene-based SNP discovery and genetic mapping in pea. Theor. Appl. Genet. **127:** 2225-2241.

Slinkard, A. E., van Kessel, C., Feindei, D. E., Aii-Khan, S. T. and Park, R. 1994. Addressing farmers' constraints through on-farm research: peas in western Canada. *In:* F. J. Muehibauer and W. J. Kaiser (eds.) Expanding the production and use of cool season food legumes. Kluwer Academic Publisher. The Netherlands. pp. 877-889.

Smith, P. G. and Walker, J. C. 1941. Certain environmental and nutritional factor affecting Aphanomyces root rot of garden pea. J. Agric. Res. **63:** 1-20.

Sparrow, F.K. 1976. A developmental study of *Achlya recurve* utilizing light and electron microscopy. M.S. Thesis. University of Texas at El Paso. 121 pp.

Statistic Canada 2011. Pulses in Canada, Canadian Agriculture at a Glance, Catalogue no. 96‑325‑X, http://www.statcan.gc.ca/pub/96-325-x/2014001/article/14041-eng.htm

Sundheim, L. and Wiggen, K. 1972. *Aphanomyces euteiches* on peas in Norway, isolation technique, physiologic races and soil indexing. Norgres Landbr. Hoiskoles Meld. **51:** 1-17.

Sundheim, L. 1972. Physiologic specialization in *Aphanomyces euteiches*. Physiol. Plant Pathol. **3:** 301-306.

Temp, M. V. and Hagedorn, D. J. 1967. Influence of cropping practices on Aphanomyces root rot potential of Wisconsin pea fields. Phytopathology **57:** 667-670.

Thabuis, A., Palloix, A., Servin, B., Daubeze, A. M., Signoret, P., Hospital, F. and Lefebvre, V. 2004. Marker-assisted introgression of four *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. Mol. Breed. **14:** 9-20.

Thygesen, K., Larsen, J. and Bodker, L. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. Eur. J. Plant Pathol. **110:** 411-419.

Trinidad, T. P., Mallillin, A. C., Loyola, A. S., Sagum, R. S. and Encabo, R. R. 2010. The potential health benefits of legumes as a good source of dietary fibre. Br. J. Nutr. **103:** 569-574.

Tu, J. C. 1987. Integrated control of the pea root rot diseases complex in Ontario. Plant Dis. **71:** 9-13.

Tu, J. C. 1990. Effect of soil pH on pea root rots, yield and soil biology. Mededelingen Faculteit Landbouwwetenschappen **55:** 827-834.

Tu, J. C. 1992. Management of root rot diseases of peas, beans, and tomatoes. Can. J. Plant Pathol. **14:** 92-99.

Tu, J. C. 1994. Effects of soil compaction, temperature, and moisture on the development of the Fusarium root rot complex of pea in southwestern Ontario. Phytoprotection **75:** 125-131.

Tu, J. C. and Findlay, W. I. 1986. The effects of different green manure crops and tillage practices on pea root rots. Proceedings of British Crop Protection Conference. **3:** 229-236.

Uzuhashi, S., Kakishima, M. and Tojo, M. 2010. Phylogeny of the genus *Pythium* and description of a new genera. Mycoscience **51:** 337-365.

Vandemark, G. J., Barker, B. M. and Gritsenko, M. A. 2002. Quantifying *Aphanomyces euteiches* in alfalfa with a fluorescent polymerase chain reaction assay. Phytopathology **92:** 265-272.

Vandemark, G., Kraft, J.M., Larsen, R., Gritsenko, M. and Boge, W. 2000. A PCR based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. Phytopathology **90:** 1137-1144.

Voglmayr, H. and Riethmüller, A. 2006. Phylogenetic relationships of *Albugo* species (white blister rusts) based on LSU rDNA sequence and oospore data. Mycol. Res. **110:** 75-85.

Wade, G. C. 1955. Aphanomyces root rot of peas – the effect of a potassium fertilizer on the severity of the disease in a potassium deficient soil. J. Austr. Inst. Agric. Sci. **21:** 260-263.

Wakelin, S. A. 2001. Biological control of *Aphanomyces euteiches* root rot of pea with spore-forming bacteria. Ph.D. Thesis, Lincoln University, Christchurch, New Zealand.

Wakelin, S., Walter, M., Jaspers, M. and Stewart, A. 2002. Biological control of *Aphanomyces euteiches* root-rot of pea with spore-forming bacteria. Australas. Plant Pathol. **31:** 401-407.

Wang, N. 2009. Quality of western Canadian peas. Canadian Grain Commission. p. 4.

Wang, S., Basten, C. J. and Zeng, Z.-B. 2012. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Watson, A., Browne, S. L., Snudden, M. G. and Mudford, E. M. 2013. Aphanomyces root rot of beans and control options. Australas. Plant Pathol. **42:** 321-327.

White, J. W., Castillo, J. A., 1992. Evaluation of diverse shoot genotypes on selected root genotypes of common bean under soil water deficits. Crop Sci. **32:** 762-765.

Wicker, E., Hullé, M. and Rouxel, F. 2001. Pathogenic characteristics of isolates of *Aphanomyces euteiches* recovered from pea in France. Plant Pathol. **50:** 433-442.

Wicker, E. and Rouxel, F. 2001. Specific behavior of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. European Journal of Plant Pathology **107**: 919–929

Wicker, E., Moussart, A., Duparque, M. and Rouxel, F. 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. Eur. J. Plant Pathol. **109:** 47-60.

Wilde, F., Schon, C. C., Korzun, V., Ebmeyer, E., Schmolke, M., Hartl, L. and Miedaner, T. 2008. Marker-based introduction of three quantitative-trait loci conferring resistance to Fusarium head blight into an independent elite winter wheat breeding population. Theor. Appl. Genet. **117:** 29-35.

Williams-Woodward, J. L., Pfleger, F. L., Fritz, V. A. and Allmaras, R. R. 1997. Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches.* Plant Soil **188:** 43-48.

Wu, L. F., Chang, K. F., Fu, H., Akter, I., Li, N., Hwang, S. F. Tumbull, G. D. and Strelkov, S. E. 2017. The occurrence of and microorganisms associated with root rot of field pea in Alberta in 2016. Can. Plant Dis. Surv. **97:** 193-195.

Xi, K., Stephens, J. H. G. and Hwang, S. F. 1995. Dynamics of pea seed infection by *Pythium ultimum* and *Rhizoctonia solani*: Effects of inoculum density and temperature on seed rot and pre-emergence damping-off. Can. J. Plant Pathol. **17:** 19-24.

Xue, A. 2003a. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. Phytopathology 93: 329-335.

Xue, A. G. 2003b. Efficacy of *Clonostachys rosea* strain ACM941 and fungicide seed treatments for controlling the root rot complex of field pea. Can. J. Plant Sci. **83:** 519-524.

Yan, W. and Kang, M. S. 2002. GGE biplot analysis of test sites: a graphical tool for breeders, geneticists and agronomists. CRC press, Boca Raton, FL, 288 pages.

Yang, J., Hu, C., Hu, H., Yu, R., Xia, Z., Ye, X. and Zhu, J. 2008. WTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics **24:** 721-723.

Yokosawa R., Kuninaga S. and Teranaka M. 1974. Note on pea root rot fungus, *Aphanomyces euteiches* Drechs. in Japan. Ann. Phytopathol. Soc. Japan **40:** 454-457(in Japanese).

Yoshida, H., Tomiyama, Y., Saiki, M. and Mizushina, Y. 2007. Tocopherol content and fatty acid distribution of peas (*Pisum sativum* L.). J. Am. Oil Chem. Soc. **84:** 1031-1038.

Zeng, Z. B. 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972-10976.

Zhang, D., Hendricks, D. G. and Mahoney, A. W. and Cornforth, D. P. 1985. Bioavailability of iron in green peas, spinach, bran cereal and cornmeal fed to anemic rats. J. Food Sci. **50:** 426-428.

Zohary, D. and Hopf, M. 1973. Domestication of pulses in the Old World. Science **182:** 887-894.