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Occurrence and Diversity of *Peronospora viciae* f. sp. *pisi* in Alberta, Canada

by

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Abstract

Downy mildew, caused by *Peronospora viciae*, is an important disease of field pea. Surveys of pea crops in central Alberta in 2009 and 2010 revealed that the incidence of downy mildew is high in this region, with yield losses of 20 to 25% in the most severely infected crop. Four pathotypes of *P. viciae* were identified when nine pathogen isolates from central Alberta were tested on a host differential set, with pathotype ABP1 found to be predominant. Random amplified polymorphic marker analysis also revealed the possibility of frequent sexual reproduction among *P. viciae* populations. A total of 81 pea cultivars and lines were assessed for downy mildew resistance under field conditions in 2008 and 2009, with 10 genotypes developing little or no disease. These results suggest that the deployment of cultivars with at least partial resistance may be an effective strategy for the management of downy mildew in Alberta.

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1.1. Pea

1.1.1. Economic significance

Pea (*Pisum sativum* L.) is an economically important annual, cool season crop that is grown worldwide. As an essential ingredient in the foods of many cultures, the demand for pea products is constant and global production is high.

According to the Food and Agriculture Organization (FAO), the five major dry pea producing countries as of 2008 consisted of Canada, followed by India, the United States, France and the Russian Federation. In 2008, Canada produced 3,571,300 metric tonnes of dry peas with an estimated value of $653,547,000. In that same year, dry pea was ranked as the 8th most economically important crop in Canada (FAO, 2010). A large proportion of the dry peas produced in Canada are exported to India, Bangladesh, Cuba and China (Anonymous, 2009). From 1999 to 2008, pea production in Alberta represented 21.2% of the national total (Wang, 2009)

1.1.2. Biology of pea

Pea is a member of the Fabaceae or legume family, a large and economically important group of flowering plants consisting of 400 genera and 10,000 species (Carr, 2006). The species of this family are found worldwide in various environments and climates.

Pea has an indeterminate growth habit. Tall cultivars are used for forage, while short cultivars with evenly maturing pods are used to produce peas for human consumption. Pea flowers are self-pollinated, and may be white, pink, purple or bi-coloured depending on the particular cultivar. The number of pods can range from 1 to 5 per node. The higher the growth density of the pea plants, the lower the number of pod-bearing nodes. The number of pods is also influenced by cultural and environmental conditions (Gane et al., 1984). The number of seeds per pod is normally 5 to 6, but can be greater depending on the cultivar and growth conditions. The dry seeds may be green, white, olive, brown or reddish-brown in color, and may sometimes have a speckled or marbled appearance. Seed weight ranges from about 90 to 400 mg per seed. Most dry seeds are round in shape, such as seeds of the cv. Midas. The majority of pea cultivars take 2 months to reach maturity after planting. Knott (1987) defined a pea developmental scale, to aid in communication between growers, researchers and other interested individuals, as well as in the timing of field operations. This scale consists of a coded key, in which pea development is divided into four main stages, namely germination and emergence, vegetative, reproductive and senescence stages. Additional secondary stages are also defined. While useful, it is not clear how widely this scale has been adopted.

1.1.3. Common diseases of pea

Peas are susceptible to infection by a number of microorganisms, including fungi, oomycetes, nematodes, viruses and bacteria. The diseases caused by these microorganisms can result, under the right conditions, in significant yield and quality

losses. Among the various pea diseases, those caused by fungi and fungal-like pathogens represent the largest group (Hagedorn and Kraft, 2000). Some of the most important of these diseases, including Ascochyta blight, powdery mildew, Fusarium wilt and downy mildew, are described below.

Ascochyta blight

Ascochyta blight is one of the most devastating pea diseases, and has been reported in many areas of the world including North America, Australia, New Zealand and Spain. This disease is caused by a complex of three fungal species: *Ascochyta pisi* (teleomorph: *Didymella pisi* sp. *nov.*), *Mycosphaerella pinodes* (anamorph: *Ascochyta pinodes*) and *Phoma pinodella* (teleomorph: *Didymella pinodella*). Among these species, *M. pinodes* is the most important pathogen and is responsible for severe yield losses, exceeding 50% in some regions (Xue et al., 1997). Given the importance of *M. pinodes* in disease development, Ascochyta blight is sometimes referred to as Mycosphaerella blight (Wallen, 1974). The anamorph of *M. pinodes*, *A. pinodes*, causes blight, while *A. pisi* causes leaf and pod spot and *P. pinodella* causes foot rot (Grunwald et al., 2004). Symptoms of Ascochyta blight can appear on infected tissues within a week of infection. Infected tissues develop dark brown, circular and necrotic lesions, which are sometimes covered with dark and globosely-shaped pycnidia. Generally, the symptoms caused by *M. pinodes*/*A. pinodes* are the most severe, although a recent report from Spain indicated that 47% to 72% of infections were caused by *A. pisi*. (Kaiser et al., 2008).

Ascochyta spp. are also effective saprophytes and can survive as thick mycelium, chlamydospores, or pycnidia (or in the case of the teleomorphic stages, perithecia) on plant debris, soil and seeds for many years. In the spring, sexual ascospores and/or asexual pycnidiospores are released from the perithecia or pycnidia, respectively, and initiate infection of the host. During the growing season, the infection is spread by secondary inoculum, which also includes pycnidiospores and ascospores. The pycnidiospores are generally dispersed by rain splash, while ascospores released from the perithecia are spread by wind currents. After harvest, the pathogen overwinters as mycelium, perithecia and pycnidia in the host debris (Schoeny et al., 2008).

The severity of Ascochyta blight is influenced by many factors, including temperature, the duration of leaf surface wetness, inoculum levels, fungal virulence patterns and host resistance. The optimum culture conditions for *in vitro* growth and sporulation were found to consist of chickpea seed meal agar (CSMA) at 15 to 20ºC (Jaiser, 1973). One study revealed that on susceptible and partially resistant cultivars, the optimum conditions for disease development included a leaf wetness period of 48 hours and an inoculum concentration of 4×10^6 resting spores/mL distilled sterilized water (Setti et al., 2008).

Management strategies for Ascochyta blight are based largely on fungicidal treatments and cultural methods, since the availability of resistant pea cultivars is limited (Zhang et al., 2006; Bretag et al., 2006; Fondevilla et al., 2008). Some effective and commonly used fungicides for seed dressing are benomyl, carbendazim, chlorothalonil, thiabendazole, thiram and mixtures of these. For foliar sprays, chlorothalonil is effective

and provides consistent control, and is the most widely used fungicide in the management of Ascochyta blight. Cultural methods such as the sowing of disease-free seeds, the burying of infested crop residues by tillage of the soil, and a 3 to 6 year rotation out of susceptible crops are helpful for reducing disease incidence and severity (Davidson and Kimber, 2007). Considerable effort has been put into breeding for Ascochyta blight resistance in pea. However, only cultivars with resistance to *A. pisi* and *P. pinodella* and with partial resistance to *M. pinodes* have been produced (Xue and Warkentin, 2001). The difficulty of developing cultivars with resistance to Ascochyta blight results from a lack of effective resistance sources and the complexity of resistance mechanisms. Gurung et al. (2002) identified resistance to the Ascochyta blight fungal complex in primitive *Pisum* and *Lathyrus* species, but the introgression of this resistance into other pea genotypes has not been successful. Bretag et al. (2006) suggested that resistance to each of the pathogens associated with Ascochyta blight is controlled by separate genes, and that resistance to foliar, stem, seed and root infections is also under separate genetic control.

Powdery mildew

Powdery mildew is caused by the obligate parasite *Erysiphe pisi* and represents the most common disease of pea. This pathogen can infect all aboveground plant tissues (Dixon 1978). *Erysiphe pisi* is an ascomycete that produces sexual fruiting bodies in the form of dark, globose-shaped cleistothecia (Kraft and Pfleger, 2001), each containing several asci. Each ascus contains eight ascospores. The anamorph or asexual stage of *E.*

pisi produces ellipsoidal to oblong conidia (13-20 × 30-50 μm in size) on conidiophores on the leaf surface. The conidia are wind-dispersed and will germinate after landing on the plant leaves, penetrating the epidermal cells and producing bipolar digitate haustoria.

The surface of infected host tissues eventually becomes covered with a dense network of hyphae and conidia interspersed with cleistothecia. Powdery mildew is characterized by the development of this whitish-gray mycelium on the surface of leaves, stems and pods of infected plants. The many conidia that are produced can give the mycelium a powdery nature, hence the name of the disease. The first symptoms usually appear on the lowest and oldest parts of the plants as small, diffuse white spots on the upper surfaces of plant tissues. As the disease develops, the mycelium may cover the entire leaf, stem or pod surface, causing discoloration. Very small dark structures may be visible within the mycelium – these are the sexual fruiting bodies or cleistothecia. Heavy infection may eventually result in the death of plant tissues (Kraft and Pfleger, 2001; Kucharek and Pernezny, 2006).

Warm, dry weather and cool nights favour the development of powdery mildew. In Canada, *E. pisi* overwinters as cleitothecia on infected plant debris or seeds. The ascospores released from the cleitothecia usually initiate the primary infections (Tiwari et al., 1999). Conidia serve as the secondary inoculum and are spread by wind over long distances. Conidia perceive contact with the plant surface within 1 minute of landing on the host (Fujita et al., 2004). High relative humidity stimulates conidial germination and favours disease development. Irrigation from the top of the plants or heavy rainfall may wash away the conidia from the surface of plant tissues and thereby reduce powdery

mildew severity (Kraft and Pfleger, 2001). The genetic mechanisms of host resistance to powdery mildew are well understood, and control is mainly through the deployment of genetically resistant pea cultivars, which are widely available (Timmerman et al. 1994; Tiwari et al. 1997; Viljanen-Rollinson et al. 1998). Other effective control methods include a rotation of 4 years out of host crops, the destruction of infected field pea debris after harvest, early sowing, and the application of foliar fungicides when disease symptoms first appear (Matthews et al., 2003).

Fusarium wilt

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *pisi*, has been reported in pea growing regions worldwide. The pathogen can survive in the soil, and in host and non-host plant debris. This wide adaptability allows for the accumulation of pathogen inoculum, which can initiate infections and cause significant yield losses, particularly under short rotations.

Eleven races of *F. oxysporum* f. sp. *pisi* have been reported, based on differences in the appearance of the fungal mycelium, pathogenicity and genomic DNA (Grajal-Martin and Muehlbauer 2002). Fusarium wilt (race 1) was first identified in Wisconsin in 1924. Diseases caused by race 2 are called near-wilts. Races 3 and 4 were reported from the Netherlands and Canada and both later re-classified as race 2. Races 5 and 6 were reported from Washington State in the 1970s. Isolates of races 1, 5 and 6 produce a mycelium that has little pigmentation, whilst mycelium of race 2 is highly pigmented when cultured on acidic potato dextrose agar. Race 2 isolates often produce asexual

fruiting bodies called sporodochia, and also tend to produce more macroconidia and microconidia than races 1, 5 and 6. The four races associated with Fusarium wilt of pea include races 1, 2, 5 and 6.

Fusarium wilt is a vascular disease. The pathogen invades through the roots, colonizing the vascular system of its host. Infected root vascular systems take on a yellowish-orange colour, which may extend up to the base of the stem. Few symptoms are visible on the root surface. Colonization of the root vascular system impedes water and nutrient uptake and transport, resulting in the abnormal growth of above-ground plant tissues. Yellowing of the leaves and a downward and inward curling of the stipules are typical above-ground symptoms of Fusarium wilt. High humidity often leads to severe infection, which can cause plant death. A white-coloured, stromatic mycelium may be visible on necrotic tissues (Sherf and MacNab, 1986).

F. oxysporum f. sp. *pisi* is an efficient saprophyte that can live in many different environments. The fungus can survive in the soil as chlamydospores for more than 10 years, and is able to live and reproduce on the roots of resistant cultivars. Infected soil, plant debris and seeds are major inoculum sources, and this inoculum can be spread by water, wind, and farm machinery. Infection of the seeds is responsible for long-distance dispersal of the fungus. Plants that are infected by race 2 isolates of *F. oxysporum* f. sp. *pisi* tend to produce internally infected seeds, since this race kills its hosts late during their life cycle (allowing the affected plants to set seed). Plants infected by isolates representing races 1, 5 and 6 tend to die before setting seed, and thus any seeds that are produced tend to be externally infested with contaminants such as soil residues and plant

debris (Kraft, 2001). There is good resistance available to Fusarium wilt of pea, and as such, this disease has become less economically important in recent years (Bodker et al., 1993; Kraft, 2001).

1.2. Downy Mildew of Pea

Downy mildew is caused by the obligate parasite *Peronospora viciae* (syn. *P. pisi*), and is one of the most common diseases of field pea. Systemic infection by the pathogen can result in stunting of the seedlings. Infection can also reduce the amount of wax on the leaf surfaces, making plants more vulnerable to herbicide damage and infection by other pathogens. High temperatures and dry weather conditions will significantly reduce downy mildew incidence and severity, and conversely, the disease is most prevalent in regions where the weather tends to be cool and moist (Dixon, 1981). Downy mildew has been reported in several major pea growing countries, including the United Kingdom, Sweden and India (Taylor et al., 1989; Stegmark, 1990; Thakur et al., 2002). In central Alberta, the disease was identified in 34 of 78 pea fields surveyed in 2006 (Chang et al., 2007). However, little is known regarding the economic impact of downy mildew in field pea.

1.2.1. Symptoms and signs of infection

A characteristic sign of downy mildew infection is the formation of a layer of grey-colored mycelium and spores on the lower leaf surface (Fig. 1-1A). On the upper

surface of the leaf, symptoms consist of yellowish-green, brownish areas and superficial blisters (Fig. 1-1B). Systemically infected seedlings are usually covered by sporulating hyphae over the entire plant surface and may be stunted in size (Fig. 1-1C). Later in the growing season, the disease may infect the flowers and pods. Infected pods have a deformed shape and are usually covered with a yellowish mycelial mass (Fig. 1-1D).

1.2.2. *Peronospora viciae* and the *Peronospora* genus

Although it has many fungal-like features, *P. viciae* is an oomycete rather than a true fungus, and therefore is classified in the eukaryotic subgroup Chromalveolates. There are about 75 species in the *Peronospora* genus, which represents the most species rich genus within the oomycetes (Anonymous, 2010). Most species in *Peronospora* are obligate plant parasites that cause downy mildew diseases in dicots (Dick, 2001).

Morphological characteristics, such as spore shape and the color and length of the terminal branches or sterigmata of the sporophores, are useful for distinguishing species with the *Peronospora* genus. *Peronospora viciae* is characterized by its tree-like conidiophores, which emerge from leaf stomata. Each hyphal branch tip bears a conidium (Thakur and Mathur, 2002) having a smooth surface during development, but which eventually becomes finely echinulate when mature (Fig. 1-1E). Conidiophores and conidia are often referred to as sporangiophores and sporangia, respectively. However, Thakur and Mathur (2002) suggested that conidiophore and conidium are more appropriate terms, since the ‘conidia’ of *P. viciae* do not produce zoospores; the term ‘sporangia’ implies the formation of additional spores, and is therefore less appropriate.

Oospores of *P. viciae* have a heavily reticulate outer wall and usually form within the infected pea pods (Falloon et al., 1996).

Morphological characteristics may not be consistent, however, because the somatic structures of plant pathogens are influenced by environmental conditions (Hall, 1996). De Bary (1863) proposed that all *Peronospora* samples infecting a specific host family be considered as a single species. More recently, molecular approaches have been employed to examine the taxonomic structure of this genus. For instance, Goker et al. (2009) outlined a taxonomical grouping of the *Peronospora* genus based on sequence analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA).

1.2.3. Pathotypes of *P. viciae*

Different races or pathotypes of a plant pathogen may evolve as a consequence of genetic variation, which in turn result from single-point mutations or sexual reproduction (Van Der Gaag and Frinking, 1996). Physiologic races or pathotypes of a parasite are identified based on their virulence patterns on a set of differential host cultivars (Parlevliet, 1985). Physiologic specialization is known to occur in *P. viciae*, and previous studies have used a variety of host differentials to identify pathotypes of this parasite. In the Netherlands, Hubbeling (1975) identified eight pathotypes of *P. viciae* based on the reaction of five groups of pea genotypes, among which the cvs. Starnain, Starcovert and Gastro were resistant to all pathotypes. In Germany, Heydendorff and Hoffmann (1978) reported four *P. viciae* pathotypes and found that the cvs. Cobri and Puget were resistant

to some pathotypes. In the United Kingdom, Taylor et al. (1989) identified 11 pathotypes of *P. viciae* based on their virulence on four pea lines: JI 411, JI560, JI758 and JI1272. Hubbeling (1975) and Taylor et al. (1989) both rated disease severity in terms of the amount of sporulation on each host line, with the aid of a five-point scale.

1.2.4. Molecular markers for evaluating genetic diversity in *P. viciae*

Little information is available regarding the genome of *P. viciae*, perhaps as a consequence of the difficulties associated with working with this obligate parasite. A recent search of the NCBI nucleotide database (J. Liu, *unpublished*) revealed only 18 entries matching *P. viciae*. The nucleotide sequences in those entries ranged from 231 to 1131 bp in length, with 14 of them corresponding to the ITS1, 5.8S and ITS2 regions of the ribosomal DNA repeat. The ITS regions are particularly informative for the analysis of genetic diversity among *Peronospora spp.* at the species level (Goker et al., 2009), but have also been used to study intraspecific variation (Feng et al., 2010).

There are many other molecular marker technologies that do not require previous knowledge of pathogen DNA sequences in order to assess genetic variation (Vos et al., 1995; Williams et al., 1990). Among the most commonly used of these techniques are amplified fragment length polymorphisms (AFLPs) and random amplified polymorphic DNA (RAPD) analysis.

The RAPD technique employs arbitrary 10 bp primers to amplify genomic DNA of each sample. The resulting PCR products are then size-fractionated by agarose gel

electrophoresis. If the genomic DNA sequences of two samples differ, then identical 10 bp primers may generate two different patterns of amplified DNA fragments. Normally, an amplicon is produced if two primers annealed close enough to each other and their 3' ends are facing each other (NCBI, 2010).

The AFLP technique uses one or more restriction enzymes to cut the genomic DNA into fragments, followed by ligation of restriction half-site specific adaptors to the sticky ends of the restriction fragments. A subset of restriction fragments is then selected for amplification with a set of primers that are complementary to the adaptor and restriction site sequences. The amplified fragments are then size-fractionated by electrophoresis on an agarose gel.

Each technique has its particular strengths and weaknesses. While the results obtained through AFLP analysis are generally more reproducible than those obtained through RAPD analysis, the former technique is more labour-intensive and expensive. RAPD markers are very quick and easy to develop, since they are based on arbitrary primers (Karp et al., 1997).

AFLPs have been used successfully to assess intraspecific variation within the *Peronospora* genus and many other fungal species (Majer et al., 1996; Rehmany et al., 2000 and Qu et al., 2008). Similarly, RAPD analysis has been used to characterize the genetic diversity of populations of a number of different types of plant pathogens, including the causal agent of downy mildew of sunflower (*Plasmopara halstedii*), the obligate parasite causing coffee rust (*Hemileia vastatrix*), and the cause of stem and root rot of soybean (*Phytophthora sojae*) (Roechel-Drevet et al., 2003; Manuela et al. 2005;

Gally et al. 2007). Given their utility in studying other downy mildews, oomycetes and obligate parasites, AFLPs and RAPD markers may represent appropriate tools to examine the genetic diversity of *P. viciae*.

1.2.5. Pathogen life cycle

The life cycle of *P. viciae* is not fully understood. Oospores serve as the primary inoculum and can remain viable in the soil for 10-15 years (Dixon, 1981). Conidia cause secondary infections and may be dispersed locally by rain splash or over longer distances by air currents. Reproduction in *P. viciae* can be sexual, resulting in the formation of oospores, or asexual, resulting in the production of conidia. Stegmark (1994) suggested that the pathogen may be both heterothallic and homothallic, and that the vegetative stage is probably diploid like other species of *Peronospora*.

The germination of *P. viciae* conidia requires high relative humidity (RH) (60-100%) (Taylor, 1989) and temperatures of 1 to 24°C (Pegg and Mence, 1970). On sorghum, Shetty and Safeeulla (1981) found that *Peronosclerospora sorghi* could produce 10,800 conidia per cm^2 of leaf area at 100% RH, 3,600 conidia per cm^2 at 85% RH, and no conidia at 80% RH. Oospores, the survival structures of *P. viciae*, are usually formed in the pods of pea when the environmental conditions are no longer favourable for pathogen growth.

Under controlled conditions (4-8°C, high RH and no light), oospores could germinate and cause systemic infection of a young plant within 4 weeks (Ryan, 1971;

Stegmark, 1991 and Van-Der-Gaag et al., 1997a). There are three methods to induce systemic infections: (1) by placing oospore inoculum just above the seeds (Ryan, 1971), (2) by spraying conidial suspensions into the apical buds of seedlings or onto the epicotyls or hypocotyls of the plants (Mence and Pegg, 1971), or (3) by soaking germinated seeds in a conidial suspension (Ryan, 1971; Stegmark, 1991).

1.2.6. Management of downy mildew

There are many possible approaches for the management of downy mildew in pea, including the cropping of genetically resistant cultivars, rotating out of susceptible crops, and the application of foliar fungicides and seed treatments. In severely infested fields, a 2-3 year rotation out of a susceptible crop is suggested, to allow inoculum levels to decline (Ocamb, 2010). The implementation of a good crop rotation is not only an economical strategy for reducing the impact of downy mildew, but is also favorable from a general pest management perspective.

The cropping of resistant pea cultivars and use of fungicidal seed treatments also represent economical options for downy mildew control. Bretag and Richardson (2010) reported the existence of a Parafield strain (P strain) and Kaspa strain (S strain) of *P. viciae* in Australia. According to this report, several commercial pea varieties, such as the cvs. Bundi, Morgan, Excell and Kaspa, are resistant to the P strain, but none are resistant to the newly found S strain. The relationship of these strains to Canadian populations of *P. viciae* is unknown, however, as is the level of resistance in Canadian pea cultivars to strains of the pathogen from this country.

Since infection of susceptible hosts is often initiated by oospores present in the soil, seed treatments such as Aliette Super® (fosetyl-aluminium 528 g/kg + thiram 172 g/kg + thiabendazole 129 g/kg) and Wakil XL (50 g/kg fudioxonil + 175 g/kg metalaxyl-M + 100 g/kg cymoxanil) have also been found to be effective for downy mildew control (Pung et al., 2005). An advantage of seed treatments over foliar fungicides is that the cost of application is considerably cheaper when using the former.

Nevertheless, if downy mildew develops in a standing crop, foliar fungicides may provide good control. Mixtures of phosphorous acid (Agri-Fos) (2000 g ai/ha) and mancozeb (Penncozeb) (1050 g ai/ha), or phosphorous acid (2000 g ai/ha) and chlorothalonil (Bravo) (1296 g ai/ha), significantly reduced disease severity in field experiments (Pung et al., 2005). Foliar fungicides may be prohibitively expensive, however, if the expected increases in returns from reduced disease pressure do not offset the cost of the chemical and its application. Regardless of the specific control measures employed, pea fields should be routinely scouted for the occurrence and severity of downy mildew, as part of a proactive pest management strategy.

1.3. Research Objectives

In Alberta, downy mildew of field pea has been observed to cause losses in some heavy infested fields, but the scope of the problem is not well understood. Therefore, the principal aim of this project was to characterize the downy mildew problem in this province. As such, there were three specific objectives: 1) to survey commercial pea fields in central Alberta and quantify the occurrence and severity of downy mildew, 2) to

study the structure of regional populations of *P. viciae* using both molecular and conventional methods, and 3) to screen a wide collection of pea cultivars and accessions for resistance to downy mildew. Through this research, we hope to better understand the extent of the downy mildew problem in Alberta, the predominant virulence patterns of *P. viceae* populations from this province, and the genetic diversity of these populations. Moreover, through the screening of pea genotypes with regional isolates of *P. viciae*, we also hope to identify good sources of effective downy mildew resistance for eventual deployment in Alberta. Studies of downy mildew have been very limited in Canada, and there are no previous in-depth studies of the disease in Alberta. An improved knowledge of the nature of downy mildew within a regional context will help in the development of effective tools and strategies to manage this disease.

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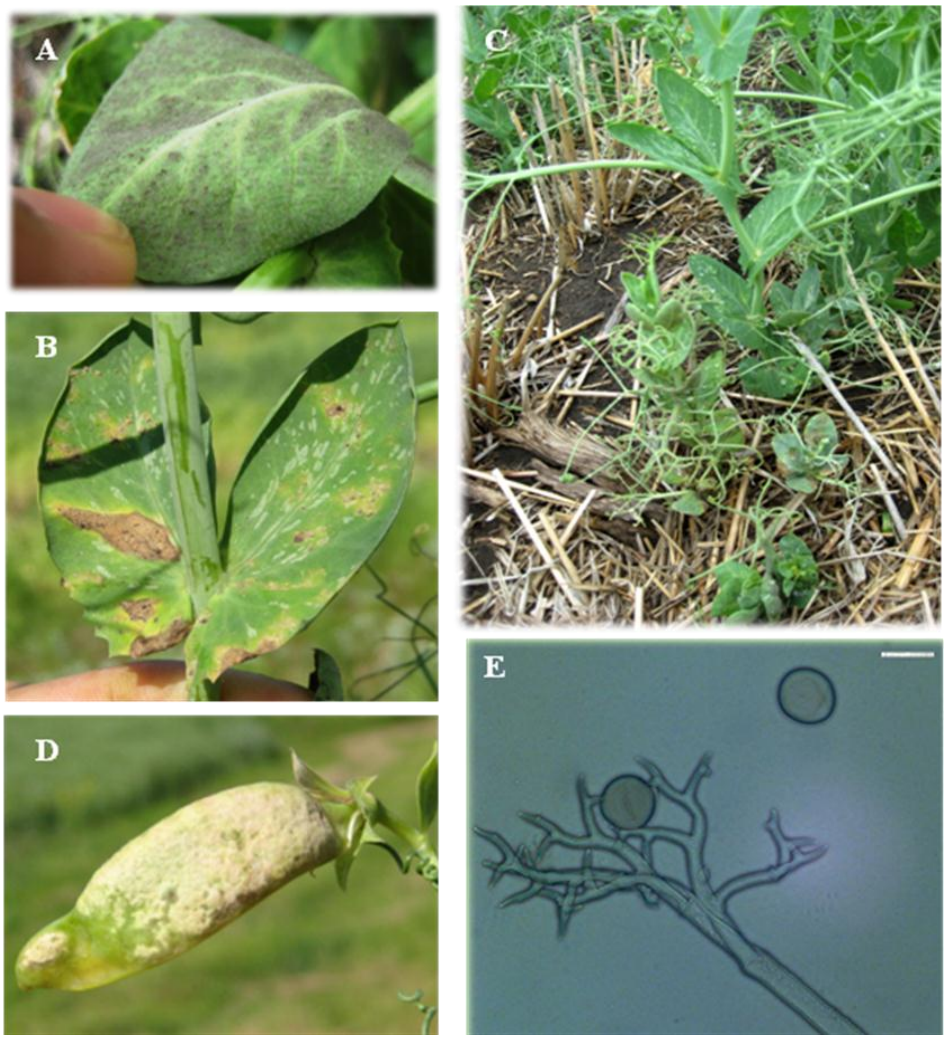

Figure 1-1. Downy mildew of field pea, caused by *Peronospora viciae*. **A**, A gray layer of *P. viciae* conidia (= sporangia) on the abaxial leaf surface; **B**, Secondary infection of field pea by *P. viciae* causing necrotic lesions; **C**, Systemically infected plants covered by a layer of gray-coloured mycelium and conidia; dwarfing and yellowing of the plants is also evident; **D**, A heavily infected pod exhibiting a deformed shape and covered by a thick layer of *P. viciae* mycelium; **E**, Conidia and tree-like conidiophore (= sporangiophore) of *P. viciae*, bar = 10 µm, magnification = 10 × 40. (All images taken by Jianfeng Liu).

Chapter 2. The Occurrence of Downy Mildew of Field Pea in Alberta

2.1. Introduction

In recent years, an increased prevalence of downy mildew was noted in pea fields in central Alberta by farmers and industry personnel working in the region. While these reports were largely anecdotal, they generally coincided with the cool, wet conditions favorable for development of this disease (Chang et al., 2008). Moreover, the diagnosis of downy mildew and its causal agent, *Peronospora viciae*, was confirmed in infected pea plants submitted for analysis to a provincial plant pathology laboratory located in Lacombe, AB (K.F. Chang, Alberta Agriculture and Rural Development, personal communication). While the occurrence of downy mildew of field pea was confirmed, the extent of the problem was not clear. As a consequence, a targeted survey was undertaken in 2008 to assess the prevalence of this disease.

In that year, downy mildew was first observed in a field pea crop near Elk Point, AB, in early June. A survey of 23 commercial pea crops was conducted in the areas around Gibbons (1 crop), Lacombe (1 crop), Mannville (9 crops), Namao (1 crop), Redwater (3 crops) and Vermilion (8 crops). Downy mildew was identified in all crops in these areas to varying extents, with the lowest disease incidence found in the crop surveyed near Lacombe (4%) and the highest (100%) in the crop near Gibbons. The average incidence of downy mildew was 58% and 50% on the crops surveyed near Redwater and Vermilion, respectively (Chang et al., 2009)

Given the results of the 2008 survey, and the fact that under the right weather conditions (low temperatures and frequent rains) downy mildew can cause significant yield losses in pea, additional surveys for this disease were undertaken in 2009 and 2010. The results from these surveys are presented in the current chapter. Surveillance for the occurrence of downy mildew of field pea represents the first step in the characterization of *P. viciae* populations from Alberta.

2.2. Materials and Methods

In 2009, a total of 37 commercial field pea crops were surveyed for the incidence and severity of downy mildew from July 15 to July 24, including 16 crops in the Mannville area, 12 crops in the Fort Saskatchewan area, and nine crops near Vermilion (Fig. 2-1). In 2010, an additional 21 commercial pea crops were surveyed for downy mildew in late June, mid-July and early August. The 2010 survey was conducted in central Alberta near the towns of Vermilion, Mannville and Namao. The incidence and severity of downy mildew was also monitored at an experimental field site in Lacombe.

Crops were surveyed at the flowering or podding stage, by examining 20 plants within a 1 m^2 area at each of five locations along the arms of a 'W' sampling pattern in each field. The presence of gray mycelial growth and conidia on the tendrils, stems and/or undersides of the leaf surfaces was taken as a sign of downy mildew infection. The incidence of disease in each field was calculated as: [(No. of infected plants / Total of plants sampled within a field)] × 100%. The severity of infection on the above-ground parts of individual plants was assessed on a 0 to 3 scale, where 0 = no infection, 1 = less

than 25% of the plant surface covered with mycelium, 2 = 25% to 50% of the plant surface covered with mycelium, and 3 = more than 50% of the plant surface covered with mycelium. Samples of infected tissue from the upper parts of the plants were collected from each field for subsequent characterization of the *P. viciae* populations as described in Chapter 3.

2.3. Results and Discussion

2009 Survey

The weather in June 2009 was hotter and drier than in previous years, creating conditions that were not conducive to downy mildew infection. As such, there were no reports of downy mildew in that month. The disease began to develop, however, after four days of continuous rain that fell in central and eastern Alberta in early July, 2009. In the survey that was conducted from July 15 to 24, downy mildew was detected in 21 of 37 commercial pea crops visited, although the severity of infection varied significantly between fields.

The most heavily infected crop, with a disease incidence of 26%, was located near Fort Saskatchewan. Yield losses in that crop as a result of downy mildew infection were estimated at 20 to 25% after harvest (Mr. Mike Kalisvaart, farmer, *personal communication*). The disease incidence in two pea crops in the Vermilion area was 13%, while it ranged from 7 to 9% in five fields visited near Mannville. In 13 of the surveyed

crops, only trace levels of downy mildew were found, with disease incidences ranging from 1% to 5%, while no disease was observed in the remaining 16 pea crops.

2010 Survey

At the Lacombe experimental site, pea line P0509-3382 was highly susceptible to downy mildew, with the development of conidia and aerial mycelia observed on the upper leaf surfaces after a period of frequent and intense rains in July and August. Infected pods were also observed on some plants. The development of signs of *P. viciae* on the upper leaf surfaces is a rare occurrence under field conditions, and likely reflected highly favourable conditions for downy mildew infection. Nonetheless, the majority of conidia and mycelia were still produced on the abaxial surfaces of the leaves. These infected plants may have provided conidial inoculum for the infection of adjacent pea lines and varieties grown at that field site. Large necrotic lesions formed on many of the infected leaves when weather conditions became hot and dry. When the pea canopy closed, however, the mature leaves were not susceptible to infection, and only the young tissues of terminal shoots developed disease. Symptoms of Mycosphaerella blight were often observed on the tendrils, and the occurrence of this disease appears to make affected plants less susceptible to downy mildew infection (K.F. Chang, personal communication). This fact, combined with differential levels of resistance, may explain the relatively low incidence of downy mildew on pea genotypes grown adjacent to line P0509-3382.

Downy mildew was also found in commercial pea crops surveyed in 2010. At Namao, one pea crop was severely infected with downy mildew (100% incidence) by mid-June. Many of the systemically infected plants were stunted and died by late July, and approximately 20% of the surviving plants developed tendril infections by early August. The incidence of downy mildew was considerably lower in pea crops in the Vermilion area, and ranged from 0 to 10%. Disease severity in this area was also low. Mycosphaerella blight was observed in every field surveyed in early August. Lodging of some of the plants was also observed in one field. In Mannville, the level of downy mildew was generally lower than in Vermilion. Most of the diseased plants exhibited systematic infections. There were few secondary infections, mostly found in the vicinity of systematically infected plants.

Impact of infection on affected plants and within-field distribution of downy mildew

On plants with a disease severity of 1 or 2, downy mildew infection had little impact on plant height, flowering, number of pods, or yield. In contrast, plants with a disease severity of 3 (severe systemic infection) were entirely covered by a gray-coloured layer of mycelia and conidia. These plants were also stunted in size. Most systemically infected plants did not flower and eventually died. Therefore, a total yield loss (100%) was observed in plants that were systemically infected. On a field scale, downy mildew generally occurred in patches, which usually corresponded to low-lying areas or parts of the field covered with crop residues and debris. This distribution likely reflected the high moisture requirements of *P. viciae*. A high incidence of downy mildew, mostly systemic

infections, was found close to the field entrances and tractor tire marks, indicating the possible spread of *P. viciae* oospores on soil carried on farm machinery. Oospores of downy mildew can survive in the soil for many years and germinate within 4 weeks under favourable conditions (Dixon, 1981).

The results from these surveys, combined with the earlier anecdotal reports of the occurrence of downy mildew, indicate that *P. viciae* is an important pathogen of field pea in central Alberta, at least when conditions are favourable for disease development. Information on the pathogenic and genotypic diversity of *P. viciae* populations from this region may serve to mitigate the impact of downy mildew, by allowing the identification and deployment of appropriate sources of genetic resistance, and is the focus of the following chapter.

2.4. Literature Cited

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Figure 2-1. Areas surveyed for the occurrence and severity of downy mildew of field pea in 2009. A total of 37 crops were visited, including 16 in the Mannville area, 12 in the Fort Saskatchewan area, and nine near Vermilion.

Chapter 3. Virulence of *Peronospora viciae* Populations from Alberta, Canada

3.1. Introduction

Downy mildew, caused by *Peronospora viciae* f. sp. *pisi*, is one of the most common diseases of field pea. *P. viciae* is an obligate parasite that is able to infect all of the aboveground parts of the plant. Substantial yield and quality losses can occur as a result of severe infection, when the entire plant becomes covered by a grey layer of mycelium (Mence and Pegg, 1971). Symptoms of downy mildew include stunted growth, distortion of the plant organs and early plant death. The incidence and severity of downy mildew often increase when the weather is cool and moist, while high temperatures and dry weather conditions are not favourable for disease development (Dixon, 1981).

Downy mildew has been reported to cause significant yield losses in various pea-growing countries. A report from Sweden indicated a yield loss in pea of up to 30% (Olofsson, 1966). In the United Kingdom, losses of 45% and 50% were observed in infected pea crops (Biddle et al., 1988; Clark et al., 2004). In Alberta, Canada, downy mildew of pea was first identified in 2004 in the central part of the province, and annual field surveys conducted from 2004 to 2008 revealed at least 57 commercial pea crops with varying levels of disease (Chang et al., 2005, 2007, and 2009). In one heavily infected crop, the yield loss was estimated at about 20-25% (Mr. M. Kalisvaart, *personal communication*). This increased prevalence of downy mildew suggests that *P. viciae* has become adapted to current field pea cropping practices and is virulent on the major pea cultivars grown in the region, including 'Midas' and 'Thunderbird'. In contrast, some

European cultivars (i.e., ‘Solara,’ ‘Eiffel,’ ‘Elan,’ and ‘Grafila’) and Australian cultivars (i.e., ‘Snopeak’ and ‘Mukta’) have been reported to be resistant to downy mildew (Sillero et al., 2006). These varieties are not currently registered or cultivated in Alberta. Given the high costs associated with chemical control of downy mildew on large field pea hectarages, the cropping of genetically resistant cultivars would represent an economically desirable strategy for disease management. Successful deployment of sources of resistance, however, requires a thorough understanding of the diversity and virulence patterns of regional populations of the pathogen (McDonald and Linde, 2002).

The occurrence of physiologic specialization in *P. viciae* has been reported by numerous researchers. In the Netherlands, Hubbeling (1975) classified *P. viciae* into five distinct pathotypes based on their virulence on a set of seven differential pea cultivars (‘Cobri,’ ‘Cicero,’ ‘Heralda,’ ‘Koroza,’ ‘Perfect Freezer,’ ‘Recette,’ and ‘Starnain’). Later, Ester and Gerlagh (1979) identified three more pathotypes using a total of 10 differential cultivars, which consisted of ‘Clause-50,’ ‘Katinka’ and ‘Puget’ in addition to the hosts of Hubbeling (1975). Heydendokff and Hoffmann (1978) reported four pathotypes of *P. viciae* in Germany using the cultivars Cobri and Puget as differentials. In Britain, Taylor et al. (1989) were able to distinguish 11 pathotypes on the cultivars Clause-50, Cobri, Katinka and Starnain. Collectively, these studies support the existence of physiological specialization in *P. viciae*, with different strains of the pathogen possessing a differential capacity to infect pea genotypes.

While there has been a fair amount of research into the virulence patterns of *P. viciae* populations, a recent literature search revealed only three molecular studies

focussed on this pathogen. One of these was a proteome-level investigation of a compatible interaction between pea and *P. viciae* (Amey et al., 2008), while two reports consisted of DNA-based taxonomic studies of *P. viciae* and its close relatives (Voglmayr, 2003; Cunnington, 2006). However, a molecular approach to investigate the genetic diversity among field populations of *P. viciae* has not been reported.

Phylogenetic analyses have been used extensively to evaluate evolutionary relatedness among fungal groups and other organisms. The internal transcribed spacer (ITS) regions are sequences separating the genes coding for the 18S and 5.8S (ITS1) and 5.8S and 28S (ITS2) ribosomal RNA (rRNA) units, and have frequently been analyzed in fungal phylogenetic studies (O'Brien et al., 2005; Sharon et al., 2008; Feng et al., 2010). The ITS sequences, which are present in high copy numbers in all eukaryotic genomes, are spliced during the transcription process and thus are not functional, exhibiting a high degree of variation even within a species (Rosenthal, 2001). Analysis of the ITS regions has been reported to be a useful method for the study of inter- and intraspecific variation (Guarro et al. 1999; Feng et al. 2010), and has been employed to assess phylogenetic relationships among species of *Peronospora* and related genera (Voglmatr, 2003). However, to our knowledge, ITS sequence-based phylogenetic analyses have not been conducted on *P. viciae*.

Random amplification of polymorphic DNA (RAPD) analysis is another technique commonly employed to assess fungal variation, and consists of the use of arbitrary 10-bp primers in a polymerase chain reaction (PCR) to amplify random segment(s) of genomic DNA from a sample (Williams et al., 1990). The resulting PCR

products are then size-fractionated by agarose gel electrophoresis. If a mutation has occurred in the template DNA at a priming site that was previously complementary to the primer, a PCR product will not be generated, resulting in a different pattern of amplified DNA fragments on the gel. RAPD analysis has been frequently used to characterize genetic variability in fungi because it is fast, inexpensive, does not involve the use of radioisotopes, and requires minimal amounts of DNA. Major polymorphisms in RAPD patterns indicate genetic distinctness, which can be used to distinguish unrelated groups or species, such as *P. viciae* and its pea host. On the other hand, minor polymorphisms may indicate genetic distinctness within groups or may occur because of experimental variability and, therefore, must be verified by repetition of the analysis. Despite its extensive application in the genotyping of plant pathogenic fungi, RAPD analysis has not been used to study the genetic variation among populations of *P. viciae* (Roeckel-Drevet et al., 2003; Manuela et al., 2005; Gally et al., 2007).

In the current study, *P. viciae*-infected shoots were collected from commercial pea crops in central Alberta, and their virulence was evaluated by inoculation of conidial suspensions extracted from these infected samples on a set of differential pea genotypes. Genetic variation among the populations was also examined via RAPD analysis and comparison of the ITS1 - 5.8S rRNA - ITS2 region. The objectives of this research were to identify the predominant *P. viciae* pathotypes in Alberta and assess genetic diversity in regional populations of the pathogen.

3.2. Materials and Methods

3.2.1. Terminology

An "isolate" refers to a collection of conidia and mycelia that were collected from a single leaf or a single plant (excluding the lower parts of the plant, which are more likely to be contaminated with other microbes) and used for DNA extraction purposes (McDonald and Linde, 2002). A "population" of *P. viciae* refers to a collection of pathogen conidia and mycelia, resulting from a mixture of two or more isolates from a given field, and used to inoculate a set of differential hosts. The term "pathotype" is used instead of "race," since the genetic uniformity of each pathogen isolate or population is uncertain (Parlevliet, 1985).

3.2.2. Pathogen collection

Thirty isolates of *P. viciae* were collected from 24 commercial pea fields in central Alberta in July of 2009 and 2010 (Table 3-1). The upper part of the infected shoots, including leaves showing obvious signs of the pathogen (grey spores and mycelium), were excised from the infected plants and collected for characterization of *P. viciae*. Briefly, the samples were sealed in plastic bags and stored at -20 °C for no more than one week prior to processing. In order to prepare the pathogen material for DNA extraction purposes, spores and masses of mycelium were gently scraped from the leaf surface with a piece of clean folded filter paper, collected in 1.5 ml Eppendorf tubes, and stored at -80 °C until DNA extraction. For host inoculation purposes, which require a

large quantity of viable conidia, nine populations of *P. viciae* were utilized, each collected from two to four infected upper shoots and representing six downy mildew infected crops near Mannville and Vegreville (see section 3.2.4 for the extraction method). During transportation from the field to the laboratory, the downy mildew infected shoots were sealed in plastic bags and stored in a plastic cooler box filled with ice.

3.2.3. Plant materials

Four pea cultivars, 'Cobri,' 'Clause-50,' 'Stamain,' and 'Katinka,' which were previously proposed by Taylor et al. (1989) as differential hosts, were obtained from the John Innes Centre (Norwich, UK). Whenever necessary, each of the four cultivars was propagated individually in a greenhouse to increase the quantity of seed stock.

3.2.4. Host inoculation, disease assessment and statistical analysis

Pea seeds were sown in 1 liter plastic pots filled with Pro-Mix potting mixture (Premier Horticulture, Rivière-du-Loup, QC, Canada) at a rate of one seed per pot. The pots were kept in a greenhouse at 24ºC (day) and 18ºC (night) with a 16 h photoperiod for two weeks, with water and fertilizer solution (1 gram per liter, 20-20-20)applied as needed.

For inoculation purposes, fresh conidia were harvested by vortexing two to four infected shoots (depending on the amount of conidia attached) with approximately 100

ml sterile distilled water (sdH_2O) in a 500 ml flask. Conidial suspensions were obtained from each population by filtering the conidial extract through two layers of cheesecloth (American Fiber & Finishing Inc., Albemarle, North Carolina). The concentration of conidia was quantified with a haemocytometer (VWR, Mississauga, ON) and adjusted to 1×10^4 to 1×10^5 conidia/ml with sdH_2O. This 10-fold variation in conidial concentration was previously tested and found to have little impact on the host reaction type (Taylor et al. 1989). Prior to spraying the suspension on the host plants, a drop of Tween 20 (polyoxyethylene sorbitol monolaurate) was added as a surfactant to enhance droplet attachment to the plant surface. The conidial suspension was sprayed onto the leaves and stems with a hand held sprayer until run-off. After inoculation, the potted plants were immediately transferred to a growth chamber and kept at 15ºC (day) and 8ºC (night) with a 16 h photoperiod and 90% relative humidity. The pots were kept saturated with water for 9 days in the growth chamber prior to disease assessment.

Nine days after inoculation, the above-ground plant tissues including leaves, stems and tendrils were examined for symptoms and/or signs of downy mildew. The severity of the infection was assessed on a 0-4 scale adapted from Taylor et al. (1989), where: 0 = no visible symptoms, 1 = local necrosis of leaves, no sporulation, 2 = limited sporulation on some leaves followed by local necrosis, 3 = abundant sporulation but confined mainly to inoculated leaves and 4 = abundant sporulation on all leaves and stems. Treatments were replicated four times, with 144 pea seedlings in total. An index of disease (ID), used to compare the overall virulence of the nine populations, was then calculated using the formula below, adapted from Strelkov et al. (2007):

$$ID(\%) = \frac{\sum(n \times 0 + n \times 1 + n \times 2 + n \times 3 + n \times 4)}{N \times 4} \times 100\%$$

Where: n is the number of plants in each class; N is the total number of plants; and 0, 1, 2, 3, and 4 are the symptom severity classes.

To compare the overall virulence of each population, the mean ID on the four host genotypes was calculated and subjected to the PROC Mixed Procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA, 2002-2003). A Tukey test was used to compare the differences among *P. viciae* populations at $P < 0.05$.

3.2.5. Common pathogens of pea

One isolate each of *Alternaria alternata*, *Ascochyta pisi*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia slcerotiorum*, and a *Pythium* species, representing common pathogens of pea, were obtained from the microorganism collection of the Plant Pathology Laboratory, University of Alberta, where they are kept under long term storage in 20% (v/v) glycerol at -80ºC. Frozen stocks were plated on 9 cm-diameter Petri dishes filled with potato dextrose agar (PDA), and grown in darkness at room temperature for one week until the mycelium covered 50-100% of the surface area of the dish. The layer of mycelium on the surface of the PDA was harvested by gently scraping it with a sterile wire loop. The colony was flooded with dsH_2O and the water decanted. The mycelial fragments in the decanted water were used for DNA extraction purposes as described below.

3.2.6. DNA extraction

Genomic DNA was extracted from spores and mycelium of *P. viciae* using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) according to the manufacturer's instructions. Prior to RAPD analysis, DNA samples were quantified using a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and normalized to 10 ng/µl. The DNA samples were stored at -20ºC when not in use. Genomic DNA was extracted from the mycelium of *A. alternata*, *A. pisi*, *B. cinerea*, *R. solani*, *S. slcerotiorum*, and *Pythium* sp. in the same manner.

3.2.7. RAPD amplification and gel electrophoresis

Eighty 10-bp oligonucleotide primers were randomly generated using a RAPD-primer generator (Wöstemeyer, 2007) available online. Only six primers that produced clear and repeatable banding patterns were screened for later applications (Table 3-2). RAPD amplification of genomic DNA was carried out in a 20-µl reaction volume consisting of 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl) (Invitrogen Life Technologies, Carlsbad, CA), 0.3 mM each dNTP, 2 mM $MgCl_2$, 0.8 U *Taq* polymerase (Invitrogen), 0.5 µM of each random primer, and 10 ng template DNA. The RAPD amplifications were carried out in a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, CA). The amplification conditions included an initial denaturation step at 94ºC for 2 min; followed by 35 cycles of 94ºC for 30 s, 35ºC for 1 min, and 72ºC for 3 min, and a final extension at 72ºC for 10 min (White et al., 1990).

PCR products (18 µl loading volume) were size-fractionated on a SYBR® Safe-stained 1.2 % agarose gel in 1× Tris-acetate-EDTA buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.3) run at 130 V for 20 min, and visualized under UV light using a Syngene BioImaging System (Syngene, Frederick, MD). In addition to DNA from the isolates of *P. viciae*, DNA from pea and *R. solani* was also included in the RAPD amplification to serve as controls.

3.2.8. Cluster analysis of *P. viciae* isolates

The RAPD fragments obtained from each *P. viciae* isolate tested were compared between pairs of isolates and scored with the Jaccard similarity coefficient (Sneath and Sokal, 1973). The Jaccard coefficient, S, was calculated as the proportion of shared DNA fragments in two isolates with the formula $S = 2N_{xy}/(N_x+N_y)$, where: N_x is the total number of fragments in isolate x, N_y is the total number of fragments in isolate y, and N_{xy} is the number of fragments common to both isolates. The distance between two isolates was calculated as $D = 1 – S$, in which D refers to the genetic distance between two isolates while S refers to the genetic similarity between two isolates. A D value of 0 indicates that the two isolates have identical RAPD fragments, whereas a value of 1.0 indicates that the two isolates have no RAPD fragments in common (Hendson et al., 2001). A binary data matrix was constructed with 0 and 1 representing the absence or presence, respectively, of a repeatable RAPD fragment observed after gel electrophoresis. A distance matrix of pairwise comparisons between isolates was constructed according to

the binary data matrix and the relationship between isolates was analyzed using the CLUSTER procedure of SAS (Statistical Analysis System; SAS Institute, Cary, NC).

3.2.9. PCR amplification, DNA cloning and sequencing

The *P. viciae* -specific primers DM3F (5´-GCCGAGTGAGCCCTATCATGGTGAGTGTT-3´) and DM3R (5´-TATGCTTAAGTTCAGCGGGTAATCTTGCCT -3´) were designed using Primer Premier Version 5.00 (Premier Biosoft International, Palo Alto, CA) and used for amplification of the partial ITS1, complete 5.8S, and partial ITS2 rRNA region of the *P. viciae* isolates (Fig. 3-1). The specificity of these primers was confirmed by using genomic DNA from pea, *Pythium* sp., *R. solani*, *A. alternata*, *B. cinerea*, and *S. sclerotiorum* as templates in the PCR. Amplifications were carried out in a 20-µl reaction volume consisting of 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl) (Invitrogen), 0.3 mM each dNTP, 2 mM $MgCl_2$, 0.8 U *Taq* polymerase (Invitrogen), 0.5 µM of each forward (DM3F) and reverse (DM3R) primer, and 10 ng template DNA. Amplification conditions included an initial denaturation step at 94ºC for 5 min; followed by 40 cycles of 94ºC for 30 s, 58ºC for 30 s, 72ºC for 50 s, and a final extension of 72ºC for 7 min. PCR products were resolved on 1% agarose gels in 1× Tris-acetate-EDTA buffer and visualized under UV light. The amplicons were then excised from the agarose gel and further purified using a GeneJET Gel Extraction Kit (Fermentas, Burlington Ontario, Canada) (Fig. 3-2). The purified DNA fragments were cloned with a pGEM®-T Easy Vector System II and multiplied in *Escherichia coli* (JM 109, Promega, USA). The

plasmids carrying the DNA fragments of interest were purified using a Qiaprep Spin Miniprep Kit (Qiagen, Mississauga Ontario, Canada).

For the sequencing reaction, amplification of purified plasmid DNA was carried out in a final volume of 20 µl consisting of 2 µl of premixed BigDye terminator reagent (Applied Biosystems, Foster City, CA), 3 µl of 5× Tris-$MgCl_2$ buffer, 1 µl of forward primer pUC/M13 5´-d(CGCCAGGGTTTTCCCAGTCACGAC)-3´ or reverse primer pUC/M13 5´-d(TCACACAGGAAACAGCTATGAC)-3´ (2.5mM) and 50 - 400 ng of plasmid DNA. Amplification conditions consisted of 30 cycles of 96^{o}C for 30 s and 60^{o}C for 2 min. The PCR products were purified and precipitated, and then sent for sequencing to the Molecular Biology Service Unit, University of Alberta, Edmonton, AB. Each plasmid sample was amplified at least twice with the pUC/M13 forward primer and pUC/M13 reverse primer for each sequencing reaction, to confirm the accuracy of each sequence.

3.2.10. DNA sequence analysis

The partial ITS1, complete 5.8S rRNA and partial ITS2 nucleotide sequences obtained were edited with BioEdit software (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html) and used in a search for homologous sequences in the National Center for Biotechnology Information (NCBI) nucleotide database (http://www.ncbi.nlm.nih.gov) using the batch BLAST program hosted at Greengene (http://greengene.uml.edu/programs/NCBI_Blast.html). The 30 ITS sequences

representing the 30 isolates were aligned using ClustalX2 (Thompson et al., 2004) for nucleotide sequence comparison.

3.3. Results

3.3.1. Virulence of *P. viciae* populations

Disease assessment after inoculation of the nine *P. viciae* populations on the four differential pea hosts resulted in IDs ranging from 6% to 88% (Table 3-3). Inoculations with the populations M10ID25, M10ID26, M11ID27, Veg1ID28, and Veg3ID31 produced IDs of 31% to 58% on 'Cobri' and 'Clause-50,' 38% to 69% on 'Starnain,' and 38% to 88% on 'Katinka' (Table 3-3). The population M11ID27 was among the populations producing the highest levels of disease, with IDs ranging from 58% to 69% on the different hosts (Table 3-3). In contrast, inoculation with the populations M9ID24, Veg2ID29, Veg2ID30, and Veg3ID32 resulted in IDs of 19% to 25% on 'Cobri,' 13% to 38% on 'Clause-50,' 19% to 56% on 'Starnain,' and 6% to 63% on 'Katinka' (Table 3-3). Veg3ID32 appeared to be a weakly virulent population, since it caused IDs that ranged only from 13% to 19% on the differential hosts (Table 3-3).

Statistical analysis using the mean ID for each pathogen population on the four differential hosts indicated significant differences in the overall levels of virulence ($P = 0.0002$). The populations M11ID27 and Veg3ID31 caused significantly higher mean IDs on all four hosts than the other populations examined (Table 3-3). In contrast, the populations Veg3ID30 and Veg3ID32 caused significantly lower mean IDs on all four

hosts. There were no significant differences in the mean IDs caused by the remaining populations (Table 3-3).

If an ID of 30% is taken as the cut-off between a resistant and a susceptible reaction, the nine *P. viciae* populations from central Alberta could be classified into four pathotypes (Table 3-3). Populations M10ID25, M10ID26, M11ID27, Veg1ID28 and Veg3ID31, which were virulent on all differentials, were grouped into pathotype ABP1, which represented the predominant strain among the characterized populations of the pathogen. Population M9ID24 was avirulent on 'Cobri' but virulent to the other three differential hosts, and was therefore classified as pathotype ABP2. Population Veg3ID32 was avirulent on all four differential hosts and classified as pathotype ABP4. Populations Veg2ID29 and Veg2ID30, collected from a pea crop near Vegreville, were only virulent on 'Starnain,' and were classified as pathotype ABP3.

3.3.2. RAPD analysis

As a consequence of the intimate contact between *P. viciae* and its pea host, and the fact that the pathogen cannot be cultured in isolation on axenic medium, it was necessary to include DNA from pea and another common fungus, *R. solani*, in the RAPD analysis, as a template to preclude the possibility of contamination. The banding patterns obtained from the host and *R. solani* were distinct from those obtained from the *P. viciae* isolates (Fig. 3-1), indicating that the *P. viciae* DNA samples were not contaminated by DNA from the host or *R. solani*.

RAPD analysis using each of the six primers generated six to eight DNA fragments from each *P. viciae* isolate, with a total of 34 clear and repeatable bands generated by all six primers. On average, each primer amplified 5.7 fragments. The amplicons were 0.5 to 7 kb in size (Table 3-2). Phylogenetic analysis using the binary data generated from the RAPD amplicons revealed three main groups: group A, (consisting of the sub-groups a1 and a2) and groups B and C (Fig. 3-2). Isolates within each group shared at least 63% genetic similarity. All nine isolates from the Vermilion area were clustered into group A, with eight of them clustering to group a2. All five isolates from the Vegreville area were clustered into group B, with at least 76% genetic similarity. With the exception of isolate FS1ID23, the four isolates from a heavily infected pea crop near Fort Saskatchewan were clustered into group a2, sharing 74% to 93% genetic similarity. The 10 isolates from the Mannville area were distributed amongst all of the groups.

3.3.3. ITS and 5.8S sequence analysis

Initially, the universal primers ITS1/ITS4 and ITS1-O/ITS4-H (Garcia-Blazquez et al., 2008) were used to amplify the ITS sequences in *P. viciae*. However, these primers were found not to be specific to *P. viciae*, as judged by the amplification of multiple bands (data not shown). Therefore, the *P. viciae*-specific primers DM3F and DM3R were designed and enabled satisfactory amplification of the ITS sequences in *P. viciae*. These primers did not amplify DNA from non-infected pea plants, *Pythium* sp., *R. solani*, *A. alternata*, or *S. sclerotiorum* (Fig. 3-3). A very weak band was produced from *B.*

cinerea, but this was larger than the product expected from *P. viciae*. A clear, single band was produced from each isolate of *P. viciae* included in the analysis (Fig. 3-4), with each of the amplicons subsequently cloned and sequenced. The corresponding sequences from the 30 isolates ranged from 724 to 728 bp in length, and contained the partial ITS1, complete 5.8S rRNA, and partial ITS2 sequences. Twenty two of the 30 sequences shared 100% homology. The sequences from the other eight isolates exhibited varying levels of variation, including several single nucleotide mutations, single nucleotide deletions, a double nucleotide deletion, and one deletion of four nucleotides (Fig. 3-5). The 5.8S rRNA region was 174 bp in length in all 30 isolates, and included base pairs 453 to 626. Relative to a 5.8S rRNA sequence from *P. viciae* found in GenBank (Accession number: AY198230.1), there were four deletions, three mutations and one insertion identified among the 30 isolates (Fig. 3-5).

3.4. Discussion

The characterization of *P. viciae* virulence patterns on the differential hosts of Taylor et al. (1989) revealed a fair amount of pathogenic diversity in parasite populations from central Alberta (Table 3-3). The pathotype ABP1, which was virulent on all four differential hosts, was predominant and found both in Mannville and Vegreville. Pathotype ABP2, which was able to cause disease on three differentials ('Clause-50,' 'Starnain,' and 'Katinka'), was found only in the Mannville area. Pathotype ABP4, collected from a pea crop in the Vegreville area, was avirulent on all hosts, and ABP3, also from Vegreville, was only virulent on 'Starnain'. While ABP3 has not been reported

elsewhere, the pathotypes ABP1, ABP2, and ABP4 were previously identified in the United Kingdom (where they were termed UKP1, UKP6 and UKP11, respectively). The occurrence of some of the same pathotypes in different regions may reflect the movement of *P. viciae*-infected seed stocks and/or similar selection pressure imposed by the cropping of the same sources of resistance. Interestingly, statistical analysis of the mean IDs caused by the nine populations on the four hosts revealed three general virulence groupings, with M11ID27 and Veg31ID31 causing significantly higher disease ('high virulence') than the other populations, Veg3ID30 and Veg3ID32 causing significantly lower disease ('low virulence'), and the remainder causing intermediate levels of disease ('intermediate virulence'). It is important to note that pathotype designations were based on the reaction of individual host genotypes to inoculation, whilst the virulence groupings were based on the mean ID on all four differentials. The virulence groupings reflect the general virulence only on the hosts evaluated.

RAPD analysis relies on randomized amplification of DNA fragments within the entire genome of a microbe, using genomic DNA isolated from pure cultures of the organism of interest. As a result of the nature of *P. viciae* as an obligate parasite, it is not possible to grow this pathogen in pure culture and the possibility exists of DNA contamination from the pea host and/or other common pea pathogens. Previously however, Morris et al. (2000) found that in a RAPD analysis of two *A. alternata* isolates possessing distinct banding patterns, the DNA from the 'contaminating' isolate was not detectable when it constituted less than 10% of the total DNA mixture. Moreover, RAPD amplification of pea and *R. solani* DNA with the same primers used for the analysis of *P.*

viciae produced very different banding patterns, suggesting that the *P. viciae* DNA was largely free of any contamination.

Phylogenetic analysis of the 30 *P. viciae* isolates, which were collected from the Fort Saskatchewan, Mannville, Vegreville, and Vermilion areas, revealed the existence of three main groups (Fig. 3-2). The fact that some of the *P. viciae* isolates collected from the Mannville area were classified into different phylogenetic groups also suggests a relatively higher degree of genetic variation in pathogen populations from this region. This may reflect the occurrence of sexual reproduction (Stegmark, 1994) and/or the widespread movement of inoculum, although more research is needed to conclusively distinguish between these possibilities. In contrast, those isolates from the Vegreville area possessed a lower degree of genetic variation relative to isolates from other regions.

Sequence analysis indicated a high level of similarity in the partial ITS1, 5.8S rRNA and partial ITS2 region of the 30 *P. viciae* isolates examined. These results suggest that comparison of this region may not be as effective as RAPD analysis for population genetics studies within *P. viciae*, likely because RAPD markers reflect genetic variation across the entire genome, while the ITS and 5.8S rRNA sequences represent a particular region of the genome. Nonetheless, the high specificity of the DM3F and DM3R primers designed in this study, along with the fact that they could amplify pathogen DNA from infected host plants (Fig. 3-3), suggests that these primers have potential as a tool for the molecular detection of *P. viciae*. Indeed, the ITS region has been a common target for the specific detection of obligate parasites such as *Plasmodiophora brassicae*, the causal agent of clubroot of crucifers (Faggian and Strelkov, 2009). Additional validation of the

primers and PCR conditions would be required, however, before they could be applied as a diagnostic technique for downy mildew under field conditions.

The current study provided the first information on the major pathotypes and population structure of *P. viciae* in central Alberta. The findings suggest a fair amount of diversity in regional populations of the pathogen, and as such, any sources of genetic resistance to *P. viciae* that are deployed in Alberta should be carefully managed. Additional research is needed, however, to fully understand the extent of downy mildew in Alberta and other pea producing areas of Canada. A larger subset of isolates should be characterized both for pathogenicity and level of genetic diversity, in order to obtain a better representation of the population structure of *P. viciae*. Ultimately, an integrated strategy, including the deployment of resistant cultivars, good crop rotation, and chemical control when necessary, may be required to effectively manage downy mildew of field pea.

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3.6. Tables

Table 3-1. Isolates of *Peronospora viciae* collected from commercial pea crops in Alberta, Canada.

Isolate	Origin	Collection Date
MID1	Mannville	7/8/2009
M2ID2	Mannville, Field2	7/23/2009
MID3	Mannville	7/8/2009
M8ID5	Mannville, Field8	7/15/2009
Ver7ID7	Vermilion, Field7	7/23/2009
FS1ID8	Fort Saskatchewan, Field1(88)	7/21/2009
FS1ID9	Fort Saskatchewan, Field1	7/16/2009
MID10	Mannville	7/15/2009
M3ID11	Mannville, Field3	7/23/2009
Ver1ID12	Vermilion, Field1	7/24/2009
Ver2ID13	Vermilion, Field2	7/24/2009
Ver3ID14	Vermilion, Field3	7/24/2009
Ver4ID15	Vermilion, Field4	7/24/2009
Ver5ID16	Vermilion, Field5	7/24/2009
Ver6ID17	Vermilion, Field6	7/24/2009
Ver7ID18	Vermilion, Field7	7/24/2009
Ver8ID19	Vermilion, Field8	7/24/2009
Ver9ID20	Vermilion, Field9	7/24/2009
FS1ID21	Fort Saskatchewan, Field1(30)	7/16/2009
FS1ID22	Fort Saskatchewan, Field1(55)	7/16/2009
FS1ID23	Fort Saskatchewan, Field1(75)	7/16/2009
M9ID24	Mannville, Field M1	7/28/2010
M10ID25	Mannville, Field M2	7/28/2010
M10ID26	Mannville, Field M2.1	7/28/2010
M11ID27	Mannville, Field M3	7/28/2010
Veg1ID28	Vegreville, Field V1	7/28/2010
Veg2ID29	Vegreville, Field V2	7/28/2010
Veg2ID30	Vegreville, Field V2.2	7/28/2010
Veg3ID31	Vegreville, Field V3	7/28/2010
Veg3ID32	Vegreville, Field V3.1	7/28/2010

Table 3-2. Oligonucleotide primers, number of amplified bands and band sizes generated with each primer in random amplified polymorphic DNA (RAPD) analysis of *Peronospora viciae* isolates from Alberta, Canada.

Primer	Sequence (5´ to 3´)	No. of bands	Size of bands (kb)
DMp4	GAAGGGTCCC	8	0.5-4
DMp6	ACGAATGGAG	4	0.8-7
DMp50	GCGCTCTTAA	3	0.5-4
DMp51	ACGCCTACCC	6	0.8-4
DMp67	CTACCCGGCT	5	0.7-4
DMp73	AACCGCTCTC	8	0.5-4

Table 3-3. Pathotype designations of *Peronospora viciae* populations from central Alberta, Canada, based on the reaction of four differential pea cultivars.

Population	Pea Cultivar[a]				Mean ID (%)[b]	Pathotype Designation[c]
	Cobri	Clause-50	Starnain	Katinka		
M9ID24	R[d] (25%)	S (38%)	S (50%)	S (63%)	44 abc	ABP2 (UKP6)
M10ID25	S (31%)	S (38%)	S (44%)	S (50%)	41 bc	ABP1 (UKP1)
M10ID26	S (42%)	S (33%)	S (50%)	S (56%)	45 abc	ABP1 (UKP1)
M11ID27	S (58%)	S (58%)	S (63%)	S (69%)	62 a	ABP1 (UKP1)
Veg1ID28	S (31%)	S (31%)	S (38%)	S (38%)	35 cd	ABP1 (UKP1)
Veg2ID29	R (25%)	R (25%)	S (56%)	R (17%)	31 cd	ABP3
Veg2ID30	R (25%)	R (25%)	S (31%)	R (6%)	22 d	ABP3
Veg3ID31	S (31%)	S (50%)	S (69%)	S (88%)	59 a	ABP1 (UKP1)
Veg3ID32	R (19%)	R (13%)	R (19%)	R (13%)	16 d	ABP4 (UKP11)

[a]The four differential pea cultivars are as per Taylor et al. (1989)

[b]ID = index of disease; mean IDs followed by the same letter are not significantly different (P = 0.0002) as assessed by the Tukey test.

[c]Pathotypes from Alberta are given the prefix "AB"; the equivalent pathotype designations from Taylor et al. (1989) are indicated in parenthese.

[d]R = resistant reaction, S = susceptible reaction; the numbers in parenthesis indicate the index of disease (ID), where an >30% is taken as a susceptible reaction.

3.7. Figures

Figure 3-1. Random amplified polymorphic DNA (RAPD) analysis of *Peronospora viciae* isolates from central Alberta, Canada. Only selected isolates of *P. viciae* are shown (Lanes 2-17) to illustrate general banding patterns. Also shown are the banding patterns obtained with pea (Lane 18) and *Rhizoctonia solani* (Lane 19). A 1 kb Plus DNA Ladder (Fermentas, Burlington Ontario Canada) was run in Lanes 1 and 20.

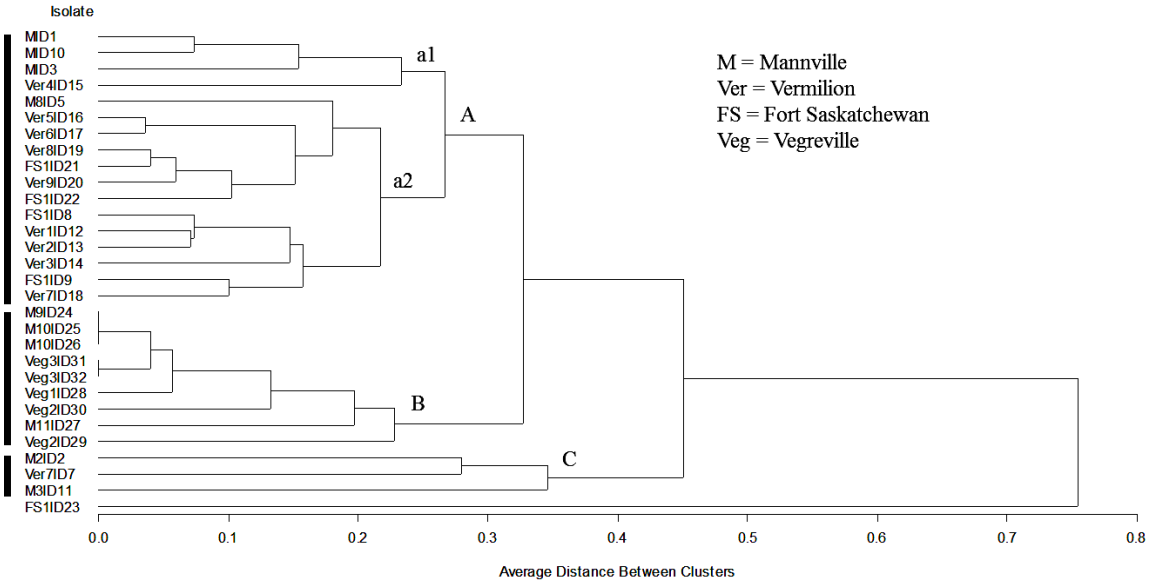

Figure 3-2. Phylogenetic tree showing genetic similarities amongst 30 isolates of *Peronospora viciae* collected from central Alberta, Canada. The tree was constructed using the pairwise comparison method according to Jaccard's distance calculation formula (SAS Institute, 1999), based on a binary data matrix with 0 and 1 representing the absence or presence, respectively, of a repeatable RAPD fragment after gel electrophoresis.

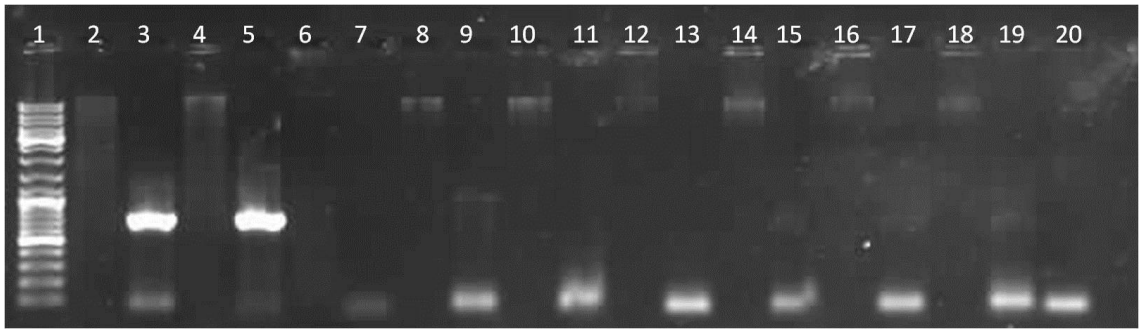

Figure 3-3. Amplification of the partial ITS1, complete 5.8S rRNA and partial ITS2 region from *Peronospora viciae*, pea and various fungal species with the *P. viciae*-specific primers DM3F and DM3R. Genomic DNA was extracted from *P. viciae*, its pea host, and various other microorganisms commonly associated with pea using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) according to the manufacturer's instructions, and used as a PCR template. Lane 1: 1 kb Plus DNA Ladder (Fermantas, Burlington Ontario Canada); Lane 2: *P. viciae* (non-amplified); Lane 3: *P. viciae* (subjected to PCR); Lane 4: *P. viciae*-infected pea leaves (non-amplified); Lane 5: *P. viciae*-infected pea leaves (subjected to PCR); Lane 6: Uninfected pea (non-amplified); Lane 7: Uninfected pea (subjected to PCR); Lane 8: *Botrytis cinerea* (non-amplified); Lane 9: *B. cinerea* (subjected to PCR); Lane 10: *Ascochyta pisi* (non-amplified); Lane 11: *A. pisi* (subjected to PCR); Lane 12: *Pythium* sp. (non-amplified) Lane 13: *Pythium* sp. (subjected to PCR); Lane 14: *Rhizoctonia solani* (non-amplified); Lane 15: *R. solani* (subjected to PCR); Lane 16: *Alternaria alternata* (non-amplified); Lane 17: *A. alternata* (subjected to PCR); Lane 18: *Sclerotinia sclerotiorum* (non-amplified); Lane 19: *S. sclerotiorum* (subjected to PCR); and Lane 20: Negative control (no DNA template).

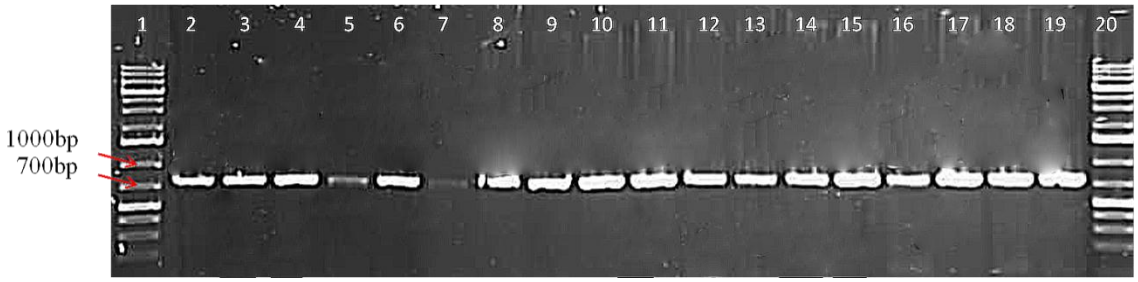

Figure 3-4. Amplification of the partial ITS1, complete 5.8S rRNA and partial ITS2 region from *Peronospora viciae* isolates from central Alberta. Genomic DNA was extracted from mycelium and spores of *P. viciae* using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) according to the manufacturer's instructions, and used as a template in PCR with the *P. viciae*-specific primers DM3F and DM3R. Lanes 1 and 20: 1 kb Plus DNA Ladder (Fermantas, Burlington Ontario Canada); and Lanes 2-19: eighteen selected isolates of *P. viciae* (another 12 isolates produced identical bands but are not shown).

```
MID1            1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
Ver7ID7         1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
FS1ID8          1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
MID10           1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
M3ID11          1   CGGGTAATCT ..-(14). ...G(190) ...A(309). A(451)GTACGGACA 460
M11ID27         1   CGGGTAATCT ..-(14). ...A(190) ...A(309). A(451)GTACGGACA 460
Veg1ID28        1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
Veg2ID30        1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
Veg3ID31        1   CGGGTAATCT ..C(14). ...G(190) ...A(309). A(451)GTACGGACA 460
EF174953       97   CGGGTAATCT ..C(110) ...G(286) ...G(405). G(547)GTACGGACA 556
AY198230.1[c]  217  ---------- -------- --------- ---------- -------TACGGACC 224
                    ITS1                                                5.8S

MID1               CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
Ver7ID7            CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
FS1ID8             CTGATA(466) ..T(489). ..T(496). .A(533).C(536) ...GTTAAA 626
MID10              CTGATA(466) ..C(489). ..T(496). .A(533).T(536) ...GTTAAA 626
M3ID11             CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
M11ID27            CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
Veg1ID28           CT----(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
Veg2ID30           CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
Veg3ID31           CTGATA(466) ..C(489). ..T(496). .A(533).C(536) ...GTTAAA 626
EF174953           CTGATA(562) ..C(593). ..C(600). .A(637).C(640) ...GTTAAA 730
AY198230.1[c]       CTGATC(230) ..C(252). ..C(259). .-(296).C(299) ...GTTAAA

MID1[a]            ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
Ver7ID7            ..AAT(680). ...T(700) ..G(703).. GATAGGGC 728
FS1ID8             ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
MID10              ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
M3ID11             ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
M11ID27            ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
Veg1ID28           ..AAT(680). ...C(700) ..G(703).. GATAGGGC 728
Veg2ID30           ..AAT(680). ...C(700) ..C(703).. GATAGGGC 728
Veg3ID31           ..G--(680). ...C(700) ..G(703).. GATAGGGC 728
EF174953           ..AAT(784). ...C(804) ..G(807).. GATAGGGC 849
AY198230.1[c]      ---------- --------- ---------- -------- 378
                   ITS2
```

Figure 3-5. Comparison of the partial ITS1, complete 5.8S rRNA, and partial ITS2 region among isolates of *Peronospora viciae* from central Alberta. The sequences obtained for isolates M2ID2, MID3, M8ID5, Ver1ID12, Ver2ID13, Ver3ID14, Ver4ID15, Ver5ID16, Ver6ID17, Ver7ID18, Ver8ID19, Ver9ID20, FS1ID21-23, M9ID24, M10ID25, M10ID26, Veg2ID29, and Veg3ID32 were identical and are represented by MID1. Two highly homologous sequences from other *P. viciae* isolates retrieved from GenBank (accession nos. EF174953 and AY198230.1) are also included for comparison.

Chapter 4. Downy Mildew Resistance in Field Pea

4.1. Introduction

Downy mildew, caused by the obligate parasite *Peronospora viciae* (syn. *P. pisi*), is an important disease of field pea that has become prevalent in central Alberta, Canada (Chang et al., 2005, 2007, 2009). The disease, which frequently occurs in cool and moist regions, affects all aboveground tissues of the plant (Dixon, 1981). On pea plants infected with *P. viciae*, the abaxial leaf surfaces and/or tendrils typically become covered with a grey layer consisting of mycelium and spores. When severe systemic infections occur the whole plant may be covered by this layer of pathogen material, resulting in stunting, tissue distortion, and early death of the infected plants. Downy mildew infection of peas can decrease yields by up to 50% under conditions favorable for disease (Clark et al., 2004).

A number of approaches are recommended for the management of downy mildew of pea, including the use of resistant host genotypes, crop rotation, fungicidal seed treatments, and the application of foliar fungicides during the growing season (Bretag and Richardson, 2010; Ocamb, 2010; Pung et al., 2005). Amongst these measures, the cropping of resistant pea genotypes is one of the most economical and environmentally friendly approaches to controlling downy mildew. Previous investigations indicated that the European cvs. Solara, Eiffel, Elan, and Grafila, as well as the Australian cvs. Snowpeak and Mukta, are resistant to downy mildew (Sillero et al., 2006). However, the resistance of most Canadian field pea cultivars has not been examined.

Complicating resistance breeding efforts, however, is the occurrence of physiologic specialization in *P. viciae* (Slep er and Poehlman, 2006). Isolates of the pathogen differ in their ability to infect specific host genotypes. Therefore, the deployment of a particular resistance source in a specific region must be guided by the knowledge of the effectiveness of that resistance against the prevalent strains of the pathogen present in the region. In Australia, for example, Bretag and Richardson (2010) reported the existence of a Parafield strain (P strain) and a Kaspa strain (S strain) of *P. viciae,* with several commercial pea genotypes ('Bundi', 'Morgan', 'Excell', and 'Kaspa') found to be resistant to the P strain, but none found to be resistant to the S strain.

Disease screening is the first step in the identification of effective downy mildew resistance for crop improvement purposes. Screening for downy mildew resistance can be carried out under field conditions or in controlled environments such as greenhouses and growth cabinets. Under field conditions, plants can be inoculated uniformly via the application of a conidial suspension onto the plant surfaces, or through the artificial inoculation of the seeds with *P. viciae* oospores prior to planting. The plants are rated a few weeks after inoculation for disease incidence and severity, providing an indication of the efficacy of the resistance present in a particular host genotype. Under controlled conditions, temperature, humidity, and light can be easily adjusted to favour the development of downy mildew, ensuring that the plants are exposed to high disease pressure. However, the results generated under such conditions may not reflect the actual degree of resistance to downy mildew under field conditions, particularly if seedlings rather than adult plants are used in the greenhouse screening. Therefore, field screening is

a necessary step to test the resistance of pea genotypes under natural conditions in a particular region.

The objective of this study was to screen a wide collection of pea genotypes in the field for resistance to *P. viciae* populations collected from Alberta, in an attempt to identify resistant genotypes that could be used as part of an integrated downy mildew management strategy in the province.

4.2. Materials and Methods

4.2.1. Field inoculation

In order to screen for downy mildew resistance in pea genotypes, field inoculations were conducted in plots located at the Lacombe Research Station, Lacombe, AB, in 2008, and in two commercial fields near Mannville, AB, in 2009. Downy mildew infected shoots, which were collected from infected crops near Vegreville, Mannville, and Vermilion, were sealed in plastic bags and stored in plastic cooler boxes filled with ice for transport to the field trials near Lacombe (in 2008) and Mannville (in 2009). At each field trial location, groups of at least four (depending on the severity of infection) diseased shoots were removed from the plastic bags and soaked in approximately 500 ml sterile distilled water (sdH_2O) in a 1000-ml flask and vigorously agitated. The pea shoots were removed and the conidial suspension was then mixed with one or two drops of Tween 20 (polyoxyethylene sorbitol monolaurate) before application as an inoculum. Inoculations were performed using a hand-squeezed bottle sprayer, and all plants within

each plot (see below) were sprayed until runoff. Inoculations were performed when seedlings were 3- to 4-weeks old (prior to the flowering and podding stages).

4.2.2. Resistance screening in 2008

In 2008, resistance screening was conducted in an experimental field which had no previous history of downy mildew and was located at the Lacombe Research Farm, Lacombe, AB. A total of 48 pea genotypes (Table 4-1) were tested in two trials (A and B); the cvs. Eclipse, Cutlass, CDC Striker, and Cooper were included as susceptible checks. Seeds of each pea genotype, were sown in soil in 5 m × 1 m plots in trial A or B, at an estimated 100 seeds per genotype per row, on May 10, 2008 using a push-seeder (Thil\ot Holland HZ423 seeder). The plots were spaced 0.6 m apart, with 2 m between blocks. The pea genotypes (plots) were arranged in a randomized complete block design (RCBD) within each block and were replicated three times. All plants of each genotype were assessed for downy mildew infection, including the number of infected shoots, in early August 2008.

4.2.3. Resistance screening in 2009

A second disease screening experiment was conducted in 2009 in a field that was previously cropped with the downy mildew susceptible genotype ‘Midas’, and which had suffered a heavy natural infestation of downy mildew in 2008. In the 2009 field trial, 40 pea genotypes were screened for downy mildew resistance in two separate trials in a field

near Mannville, AB. Of the 40 genotypes evaluated, 25 were kindly donated by Dr. D. J. Bing (Research Scientist, Agricultural and Agri-Food Canada, Lacombe, AB) while the rest were commercially purchased. The 25 pea genotypes from Dr. Bing were included in trial A, and the 15 commercially purchased genotypes were included in trial B. The pea genotypes (plots) in each trial were arranged in a RCBD and were replicated 4 times. Each plot was sown in two 6 m rows with 30 cm row spacing, 45 cm between plots and 1 m between blocks. Approximately 100 seeds were sown per row with a push-seeder on May 13, 2009. 'Midas' was included as a susceptible check in trial A, while 'Agassiz' and 'Thunderbird' were included as checks in trial B.

For weed control, the herbicides Pursuit (imazethapyr) and Poast Ultra (Sethoxydim) were applied at rates of 210 ml/ha and 470 ml/ha, respectively, on June 12, and Basagran Forte (bentazone) was applied at a rate of 2237 ml/ha on July 2. To desiccate the crop in order to facilitate the harvest, the herbicide Reglone [6,7-dihydrodipyrido(1,2-a:2',1'-c)pyrazinediium dibromide] was applied at a rate of 1852 ml/ha on August 25, 2009.

At the podding stage, the number of systemically infected plants and/or dead plants (all tissue dried up, dark in color, distorted and covered with a grey layer of mycelium and spores) was recorded for each plot. In addition, the seeds of all the 40 genotypes were harvested manually on September 5, 2009. The seeds were dessicated in an incubator at 40 °C for 48 hours, cleaned with a seed cleaner (Almaco Air Blast Seed Cleaner, Nevada, Iowa), and weighed.

4.2.4. Statistical Analysis

In the analysis of trials A and B in 2008, the independent variable was pea genotype. The response variables, number of plants that had stem/leaf, tendril and pod infections, were recorded and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA, 2002-2003). A test of Fisher's Least Significant Difference (LSD) was used to compare the differences among treatments at $P < 0.05$. Pearson's correlations among stem/leaf, tendril and pod were calculated using Minitab Statistic Software v. 14 (Minitab Inc., USA, 1972-2004)

In the analysis of trials A and B in 2009, the independent variable was pea genotype. The response variables, yield and mortality rate, were collected and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA, 2002-2003). A test of Fisher's Least Significant Difference (LSD) was used to compare the differences among treatments at $P < 0.05$. Pearson's correlations between yield and mortality were calculated using Minitab Statistic Software v. 14 (Minitab Inc., USA, 1972-2004)

4.3. Results

4.3.1. Resistance screening in 2008

In trial A of the 2008 experiment, low levels of tendril infection were observed on the susceptible check genotypes 'Eclipse' (0.3%), 'Cutlass' (0.7%), and 'Cooper' (0.3%), while no signs or symptoms of downy mildew infection were observed on the fourth

check, ‘CDC Striker’ (Table 4-2). Of the host genotypes being assessed for their reaction to downy mildew, LAN1101, LAN4188, LAN4190, CDC1872-207 and MP1856 did not show any symptoms of infection, while LAN1104 exhibited the highest infection rate, with 23.3% tendril infection. Genotypes LAN 4194, LAN1103, CDC 1897-14, CDC 1897-3, CDC 1908-28, CDC1932-201, CDC2093-22, CDC 2235-4, CDC 2046-4, CDC 2237-7, CDC 123-10, CDC 1996-216, MP 1861 and MP 1867 exhibited relatively low levels of infection, with tendril infection rates ranging from 0.3% to 4.6% (Table 4-2). In most genotypes tested, higher infection rates were observed on the tendrils than on the stems, leaves or pods. In CDC1932-201, CDC2235-4 and CDC2237-7, however, infection rates were 0.3%, 1.7% and 4% on the stems/leaves versus 0%, 0% and 3.7% on the tendrils, respectively (Table 4-2). The highest levels of stem/leaf infection among the 24 genotypes assessed in trial A of the 2008 experiment were observed on LAN1104 and CDCN 2237-7 (Table 4-2). No correlations were observed between stem/leaf and tendril, stem/leaf and pod, or tendril and pod for each genotype ($P = 0.702$, 0.660 and 0.845).

In trial B of the 2008 experiment, all susceptible checks were infected with downy mildew, with 2.7% of ‘CDC Striker’, 1.3% of ‘Cutlass’ and 0.7% each of ‘Eclipse’ and ‘Cooper’ plants exhibiting tendril infections (Table 4-3). The genotypes LAN4201, CDC2096-11 and APCM71.13 appeared the most susceptible, with tendril infection rates of 12%, 11% and 9%, respectively. The tendril infection rates on the remainder of the genotypes ranged from 0 to 6.7%, among which genotypes MP1860 and MP 1862 exhibited no symptoms or signs of infection (Table 4-3). As in trial A, tendril infection was more common than infection of the stems, leaves or pods in most genotypes in trial B. The exceptions were genotypes JSA3019.3, APCM07602, APCM25430, ‘Alezan’,

and CDC1861-16. In the case of JSA3019.3, 'Alezan' and APCM25430, there were more plants with pod infections than tendril or stem/leaf infections, while in the case of APCM07602 and CDC1861-16, there were more plants with stem/leaf infections than tendril or pod infections (Table 4-3). No correlations were observed between stem/leaf and tendril, stem/leaf and pod, or tendril and pod for each genotype (P = 0.811, 0.247 and 0.290).

4.3.2. Resistance screening in 2009

In trial A of the 2009 experimental year, all pea genotypes tested showed at least some signs and/or symptoms of downy mildew infection. The lowest mortality rate was observed on genotype MP1868, with 5% of plants systemically infected or dead. The highest mortality rates were observed on the susceptible check cv. Agassiz with 35% of plants systemically infected or dead, followed by MP 1875 (31%), MP1867 (27%), MP1872 (23%), and MP1878 (21%) (Table 4-4). There were no significant differences in mortality rate between MP 1868 and genotypes MP1861, MP1862, MP1864, MP1866, MP1869, MP1870, MP1871, MP1873, MP1874, MP1876 to MP1882, 'Thunderbird', MP1846, P0509 and 'Midas', for which the percentage of systemically infected or dead plants ranged from 8% to 19% (Table 4-4). With respect to yield assessments, the check genotype MP1846 produced the highest yield at 441.9 g of seeds per single row plot, followed by MP1870 and MP1881 at 408.7 g and 406.8 g, respectively. The lowest yield was observed for the check genotype P0509, at 216.8 g of seeds per single row plot. The remaining genotypes produced yields that ranged from 226.9 g (MP1871) to 387.4 g per

single row plot (MP1869) (Table 4-4). No correlations were observed between yield and mortality rate for each genotype (P = 0.155).

In trial B of the 2009 experiment, the lowest mortality rate was observed for 'Circus', at 3.5%, followed by 'Stratus', 'Noble', and 'Cooper', at 4%, 4% and 5%, respectively. The highest mortality rates were observed for 'Cutlass' and 'Midas' at 23% and 22.5%, respectively. The mortality rates of the remaining genotypes ranged from 7% to 20.5% (Table 4-5). The highest yield was observed on 'Golden' at 309.3 g of seeds per single row plot, followed by 'Capri' and 'Marquee' at 301.9 and 300 g, respectively. The other genotypes produced yield that ranged from 138.2 g ('Camry') to 285.7 g ('CDC Striker') of seeds per single row plot (Table 4-5). No correlations were observed between yield and mortality rate for each genotype (P = 0.959).

4.4. Discussion

Given the increased occurrence of downy mildew in central Alberta in recent years, it appears that *P. viciae* has become well adapted to current field pea production systems in the region. Unfortunately, the major pea cultivars grown in Alberta appear to be susceptible to *P. viciae* infection (Chang et al., 2005, 2007, 2009), further complicating control of the disease. The aim of the current study was identify potential sources of resistance against the regional populations of *P. viciae*, since pea genotypes with effective resistance would represent an important tool for the management of downy mildew.

To our knowledge, none of the 81 pea genotypes examined in this study had previously been assessed for downy mildew resistance. However, the cv. Midas, which is one of the most widely grown genotypes in Alberta, was previously observed to be heavily infected in commercial fields (Chang et al., 2005, 2007, 2009). In trial B of the 2009 experiment, 'Midas' had the second-highest mortality rate (22.5%) of all the genotypes tested, confirming its susceptibility to the strains of *P. viciae* in Alberta.

The field screening trials in 2008 and 2009 revealed that there are some genotypes with good levels of resistance to downy mildew. Amongst the 44 genotypes tested in 2008, five genotypes (LAN1101, LAN4188, LAN4190, CDC 1872-207 and MP1856) did not have any visible signs or symptoms of downy mildew infection (Tables 4-2 and 4-3). The genotype MP1846 (2009 trial A) produced the highest yield among all of the tested genotypes, while MP1868 (2009 trial A) and 'Circus' (2009 trial B) exhibited the lowest mortalities in response to downy infection (Table 4-4 and Table 4-5), suggesting that those three genotypes may perform best under conditions in which downy mildew is prevalent. However, caution should be used in interpreting data from field screening experiments, as disease pressure may vary from year to year. The development of downy mildew is affected both by weather conditions during the growing season, as well as by the distribution of the pathogen within and between fields.

Cool, wet weather favours disease development, whereas drier and hotter weather does not (Dixon, 1981). In 2009, downy mildew did not occur until early July, following 5 days of continuous rain and wet conditions. Young seedlings exhibited systemic infections, but infected plants were unevenly distributed in the field. Systemically

infected plants died approximately 3 weeks after the appearance of the first signs and symptoms of infection. The presence of abundant signs of *P. viciae* (i.e., copious amounts of conidia and a layer of greyish coloured mycelium) enabled identification of downy mildew as the cause of plant death as opposed to other pathogens, such as *Rhizoctonia solani*, which cause root rots.

Given its nature as a soilborne pathogen, the occurrence of *P. viciae* within a field may also be patchy. Oospores, which are sexual resting spores that can serve as primary inoculum for the pathogen, are often not uniformly distributed in the soil, resulting in zones of higher or lower disease pressure. For example, the check genotypes 'Eclipse', 'Cutlass', 'CDC Striker', and 'Cooper' exhibited higher infection rates in trial B than in trial A in 2008. Since the trials were conducted simultaneously and within close proximity to each other, this difference in the amount of disease probably reflected the differential concentration of oospores in the soil in different parts of the field. The trials in 2009 were conducted in a field that had seen heavy levels of downy mildew in the previous year, and the application of pathogen conidia as additional inoculum at the start of the 2009 trial was probably insufficient to compensate for these differences in the amount of oospores. Earlier field surveys (Chapter 2) also revealed that within a field, downy mildew generally occurred in patches, which usually corresponded to low-lying areas or parts of the field covered with crop residues and debris.

In the future, resistance screening should also include a component of screening under controlled environmental conditions, such as in a greenhouse or growth cabinet, in order to confirm the resistance of the above genotypes to *P. viciae*. This would allow

screening against characterized pathogen of the pathogen, such as those representing predominant pathotypes (Sillero et al., 2006), as well as ensure favourable conditions for infection.

It is unknown whether the resistance of pea to *P. viciae* is polygenic or oligogenic. In an earlier study, Clark and Spencer (1994) identified a partially resistant genotype, 'Early Onward', which suppresses *P. viciae* sporulation on leaflets and seedling shoot apices. In the current study, several genotypes (MP1846, MP1870, and MP1881) developed little or no downy mildew while producing fairly good yields, suggesting that the deployment of these genotypes with at least partial resistance may be an effective strategy for the management of downy mildew in Alberta.

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4.6. Tables

Table 4-1. List of pea genotypes evaluated in field trials conducted in central Alberta in 2008 and 2009.

Genotype	Year tested*	Genotype	Year tested*
Eclipse	2008AB	MP 1862	2008B, 2009A
Cutlass	2008AB	MP 1863	2008B
CDC Striker	2008AB	MP 1864	2008B, 2009A
Cooper	2008AB	MP1861	2009A
LAN 1101	2008A	MP1866	2009A
LAN 4188	2008A	MP1867	2009A
LAN 4190	2008A	MP1868	2009A
LAN 4194	2008A	MP1869	2009A
LAN 1103	2008A	MP1870	2009A
LAN 1104	2008A	MP1871	2009A
CDC 1897-14	2008A	MP1872	2009A
CDC 1897-3	2008A	MP1873	2009A
CDC 1908-28	2008A	MP1874	2009A
CDC 1872-207	2008A	MP1875	2009A
CDC 1932-201	2008A	MP1876	2009A
CDC 2093-22	2008A	MP1877	2009A
CDC 2235-4	2008A	MP1878	2009A
CDC 2046-4	2008A	MP1879	2009A
CDC 2237-7	2008A	MP1880	2009A
CDC 123-10	2008A	MP1881	2009A
CDC 1996-216	2008A	MP1882	2009A
MP 1856	2008A	Agassiz	2009A
MP 1861	2008A	Thunderbird	2009A
MP 1867	2008A	MP1846	2009A
JSA 3019.3	2008B	P0509	2009A
APCM 71.13	2008B	Midas	2009AB
APCM 07602	2008B	Camry	2009B
APCM 25430	2008B	Striker	2009B
LAN 4193	2008B	Sage	2009B
LAN 4200	2008B	Cooper	2009B
LAN 4201	2008B	Nitouche	2009B
LAN 4202	2008B	Stratus	2009B
LAN 4195	2008B	Capris	2009B

Alezan	2008B	Bronco	2009B
CDC 1329-12	2008B	Golden	2009B
CDC 1861-16	2008B	Cutlass	2009B
CDC 1897-2	2008B	Admiral	2009B
CDC 2096-11	2008B	Circus	2009B
Partner	2008B	Marquee	2009B
MP 1859	2008B	Noble	2009B
MP 1860	2008B		

*A = 2008 trial A or 2009 trial A; B: 2008 trial B or 2009 trial B;

Table 4-2. Response of 24 pea genotypes to downy mildew in trial A under field conditions at Lacombe, Alberta, in 2008.

Genotype	Plants infected (%)*		
	Stem/Leaf	Tendril	Pod
Eclipse	0 c	0.3 c	0 c
Cutlass	0 c	0.7 c	0 c
CDC Striker	0 c	0 c	0 c
Cooper	0 c	0.3 c	0 c
LAN 1101	0 c	0 c	0 c
LAN 4188	0 c	0 c	0 c
LAN 4190	0 c	0 c	0 c
LAN 4194	0 c	0.7 c	0 c
LAN 1103	1.0 bc	4.6 b	0.7 bc
LAN 1104	3.7 a	23.3 a	1.7 a
CDC 1897-14	0.3 bc	1.7 bc	0.3 bc
CDC 1897-3	0 c	0.3 c	0 c
CDC 1908-28	0 c	0.7 c	0.3 bc
CDC 1872-207	0 c	0 c	0 c
CDC 1932-201	0.3 bc	0 c	0 c
CDC 2093-22	0 c	2.7 bc	0 c
CDC 2235-4	1.7 b	0 c	0 c
CDC 2046-4	1.0 bc	1.3 bc	1.0 ab
CDC 2237-7	4.0 a	3.7 bc	0.3 bc
CDC 123-10	0 c	0.7 c	0 c
CDC 1996-216	0 c	1.7 bc	0 c
MP 1856	0 c	0 c	0 c
MP 1861	0 c	0.3 c	0.3 bc
MP 1867	0.3 bc	0.3 c	0 c

* Different letters within the same column indicate significant differences based on Fisher's least significant difference at $P < 0.05$.

Table 4-3. Response of 24 pea genotypes to downy mildew in trial B under field conditions at Lacombe, Alberta, in 2008.

Genotype	Plants infected (%)*		
	Stem/Leaf	Tendril	Pod
Eclipse	0 b	0.7 c	0.3 bc
Cutlass	0.3 b	1.3 c	0.3 bc
CDC Striker	0.7 b	2.7 bc	0.3 bc
Cooper	0 b	0.7 c	0 c
JSA 3019.3	0 b	2.3 bc	4.3 a
APCM 71.13	1.0 b	9.0 ab	0 c
APCM 07602	0.3 b	0.3 c	0 c
APCM 25430	0 b	0.3 c	1.3 bc
LAN 4193	0 b	1.3 c	0 c
LAN 4200	0 b	1.7 c	1.0 bc
LAN 4201	3.3 a	12.0 a	2.7 ab
LAN 4202	0.3 b	3.3 bc	0 c
LAN 4195	0 b	1.0 c	0 c
Alezan	0 b	0.3 c	0.3 bc
CDC 1329-12	0 b	0.3 c	0 c
CDC 1861-16	0.7 b	0.3 c	0 c
CDC 1897-2	0 b	2.7 bc	0 c
CDC 2096-11	5.0 a	11.0 a	0.7 bc
Partner	0 b	0.3 c	0 c
MP 1859	0.3 b	6.7 abc	0.3 bc
MP 1860	0 b	0.3 c	0 c
MP 1862	0 b	0.3 c	0 c
MP 1863	0.7 b	5.3 abc	0.3 bc
MP 1864	0 b	4.0 bc	0 c

* Different letters within the same column indicate significant differences based on Fisher's least significant difference at $P < 0.05$.

Table 4-4. Response of 25 pea genotypes to downy mildew in trial A under field conditions at Mannville, Alberta, in 2009.

Genotype	Yield (g seed/single row plot)*	Mortality rate (%)*
MP1861	331.9 abcdef	11 cde
MP1862	350.6 abcdef	18 bcde
MP1864	233.6 def	16 bcde
MP1866	284.0 bcdef	10 de
MP1867	318.7 abcdef	27 abc
MP1868	298.1 abcdef	5 e
MP1869	387.4 abc	12 cde
MP1870	408.7 ab	15 cde
MP1871	226.9 ef	18 bcde
MP1872	278.9 bcdef	23 abcd
MP1873	249.3 cdef	13 cde
MP1874	380.4 abcd	15 cde
MP1875	309.7 abcdef	31 ab
MP1876	274.4 bcdef	15 cde
MP1877	294.1 abcdef	14 cde
MP1878	275.2 bcdef	21 abcde
MP1879	333.4 abcdef	11 cde
MP1880	296.3 abcdef	14 cde
MP1881	406.8 ab	8 de
MP1882	371.2 abcde	14 cde
Agassiz	253.6 cdef	35 a
Thunderbird	324.6 abcdef	8 de
MP1846	441.9 a	13 cde
P0509	216.8 f	15 cde
Midas	290.4 bcdef	19 bcde

* Different letters within the same column indicate significant differences based on Fisher's least significant difference at $P < 0.05$.

Table 4-5. Response of 15 pea genotypes to downy mildew in trial B under field conditions at Mannville, Alberta, in 2009**.**

Genotype	Yield (g seed/single row plot)*	Mortality rate (%)*
Camry	188.2 d	10 abc
Striker	285.7 ab	7 bc
Sage	245.8 abc	15.5 abc
Cooper	188.0 bcd	5 c
Nitouche	236.9 abcd	14 abc
Stratus	180.0 bcd	4 c
Capris	301.9 a	9.5 abc
Bronco	275.8 abc	15 abc
Golden	309.3 a	11 abc
Cutlass	260.8 abc	23 a
Admiral	238.8 abc	20.5 ab
Circus	206.5 abcd	3.5 c
Midas	178.3 cd	22.5 a
Marquee	300.0 a	8 abc
Noble	282.0 ab	4 c

* Different letters within the same column indicate significant differences based on Fisher's least significant difference at $P < 0.05$.

Chapter 5. Summary and Conclusions

5.1. Downy Mildew of Field Pea in Alberta

In recent years, anecdotal reports from farmers and other agricultural personnel suggested an increasing prevalence of downy mildew in pea crops in central Alberta. These reports were confirmed by annual surveys conducted for downy mildew, which revealed that the disease is common in this region (Chang et al., 2005, 2007, 2009; Chapter 2). Proper management of downy mildew and other diseases requires a good understanding of the causal agent and its characteristics. As such, the work presented in this thesis was aimed at increasing this understanding, and identifying effective sources of resistance against *P. viciae*.

Physiologic specialization and genetic diversity in *P. viciae* were investigated in order to find the predominant virulence patterns of pathogen populations in Alberta. Pathotype characterization studies revealed the presence of four pathotypes among the nine populations tested, based on their virulence on four pea differential hosts proposed by Taylor et al. (1989) (Table 3-5). ABP1, which was virulent on all four differentials, appeared to be the predominant pathotype in Alberta. ABP2, which was virulent on three of the four pea differentials, and ABP4, which was avirulent on all of the differentials, were also present in the province. A pathotype equivalent to ABP3 was not previously identified by taylor et al. (1989), when they used the same differentials to characterize *P. viciae* populations from the United Kingdom. The RAPD analysis conducted in Chapter 3 suggested that *P. viciae* isolates collected from pea crops near Vermilion and Fort

Saskatchewan were most closely related to each other, while isolates collected from the Mannville area showed the highest diversity. This analysis also revealed the possibility of frequent sexual reproduction among pathogen populations.

A number of pea genotypes, which are either research breeding lines or commercially available cultivars, were assessed under field conditions for their resistance to downy mildew (Table 4-1). The pea genotypes LAN1101, LAN4188, LAN4190, CDC 1872-207, MP1856 in the 2008 trials developed no disease and the genotypes MP1846, MP1870, and MP1881 in the 2009 trials, developed little disease while producing fairly good yields, suggesting that the deployment of cultivars with at least partial resistance may be an effective strategy for the management of downy mildew in Alberta.

5.2. Future Studies

Additional research is needed to better understand the extent of the downy mildew problem in Alberta and other pea producing regions of Canada. This will require continued surveillance for downy mildew. Moreover, since the number of *P. viciae* isolates examined in the current work was fairly limited, a better indication of pathogen diversity and virulence phenotypes may result from the characterization of additional isolates and populations. It would also be useful to obtain isolates previously described from other parts of the world, such as the United Kingdom (Taylor et al., 1989) for comparison with regional populations of *P. viciae*. The inclusion of such reference isolates would facilitate research into the origin of regional populations of the pathogen, as well as help in identifying potentially useful sources of resistance. For studies of the

pathogen in a Canadian context, it may also be useful to include some regional pea genotypes in the host differential set. The commonly grown cv. Midas, which appeared to be highly susceptible to downy mildew in Alberta, could serve as a susceptible check. In addition, it would be helpful to include a highly resistant pea genotype as a differential; erosion or breakdown of the resistance in such a host could then serve as an indication of a pathotype shift in the *P. viciae* population.

In the resistance screening study (Chapter 4), seven pea genotypes (including LAN1101, LAN4188, LAN4190, MP1856, MP1860 and MP1862) were found to be free of downy mildew infections, suggesting superior resistance relative to the rest of the genotypes assessed. Those genotypes that performed well may be useful for the development of downy mildew resistant pea cultivars for western Canada. From an academic perspective, studies into the inheritance of this resistance may reveal whether or not it is under monogenic or oligogenic control. This would not only be useful information for resistance stewardship purposes, but would also improve understanding of host-pathogen interactions in the downy mildew pathosystem.

While resistance in the field represents the ultimate litmus test for material that may eventually see commercial deployment, future resistance screening efforts should also include a component of screening under controlled environments. This would allow researchers to screen with pathotypes of *P. viciae* that may be of particular interest, such as those that are predominant within a particular region. Furthermore, environmental conditions and pathogen inoculum levels can be manipulated in a greenhouse or growth cabinet, so as to ensure sufficient disease pressure to make accurate assessments

regarding resistance. This is not always possible in the field. A general approach might be to first conduct a screen under greenhouse conditions, followed by field screening of only those host genotypes that performed well in the greenhouse (S.E. Strelkov, *personal communication*). This approach would also reduce the space and labour requirements associated with field screening.

Downy mildew has emerged as an important disease of field pea in central Alberta. Hopefully, the work presented in this thesis, along with other research being conducted in the province and elsewhere, will increase our knowledge and ability to manage this disease. Ultimately, it is likely that an integrated approach, including genetic resistance, appropriate cultural practices and chemical fungicides, will be required to successfully minimize the impact of downy mildew on the production of field pea.

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