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Morphological characterization of fungi associated with the ascochyta blight complex and pathogenic variability of *Mycosphaerella pinodes* on field pea crops in central Alberta

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ABSTRACT

Field pea crops in central Alberta were surveyed for ascochyta blight from 2011 to 2012 and fungal isolates were recovered from foliar lesions on selected plants. Cultural and microscopic characterization of the 275 isolates obtained revealed that 272 were of *Mycosphaerella pinodes* and three were of *Phoma medicaginis* var. *pinodella*. *Ascochyta pisi* or *Phoma koolunga* were not identified. Isolates of *M. pinodes* were divided into two groups, GI and GII, based on visual assessment of culture characteristics. GI isolates (light to dark, mostly gray colony color; pycnidial distribution radial and concentric; conidia 10.5–14.5 × 4.2–6.2 μm most with one septum, occasionally two, constricted at the septum; spore mass light buff to flesh color) were predominant (83%), while GII isolates (dark to gray colony color; pycnidia abundant; conidia 8–16 × 3.5–6.2 μm most with 1 septum, constricted at the septum; spore mass light buff to flesh color) were less common (17%). The cultures of GII isolates were similar to recent descriptions of *A. pisi*, but they differed in spore color. In a host differential study, 13 pathotypes of *M. pinodes* were identified from 110 single-spore isolates. Pathotype I was predominant (88 isolates) and virulent on all nine differential genotypes. The other pathotypes (pathotypes II–XIII) were rare (1–6 isolates of each). Comparison of the present results with earlier studies suggests that pathotype I has been prevalent for many years, and that its aggressiveness on the host differentials has increased over time. Emphasis should be placed on breeding for resistance to *M. pinodes* in field pea cultivars intended for deployment in central Alberta.

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1. Introduction

Ascochyta blight of pea is a disease complex involving the fungal pathogens *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr, (anamorph *Ascochyta pinodes*), *Ascochyta pisi* Lib., and *Phoma medicaginis* var. *pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch. Recently, *Phoma koolunga* Davidson et al. sp. nov. has been identified as an important component of the blight complex on field pea (*Pisum sativum* L.) and has become widespread in South Australia [1,2]. In Canada, the ascochyta blight complex is also a serious impediment to field pea production, but the association of *P. koolunga* with the blight complex is not yet known. All of these fungi can commonly be isolated from the same plants or from the same or adjacent lesions on the leaves, pods and stems [1–3]. *Mycosphaerella pinodes*, *A. pisi*, *P. medicaginis* var. *pinodella* and *P. koolunga* are all seed borne pathogens that can also survive on infected pea debris [2,4,5]. Mycosphaerella blight can cause substantial damage to field pea, with estimated losses of 10% in commercial crops and greater than 50% in field trials [6,7].

Mycosphaerella pinodes can infect seedlings and all above-ground parts of adult pea plants, causing foot rot of seedlings, necrotic leaf spots, stem lesions and blackening of the base of the stem, as well as shrinkage and dark-brown discoloration of seed. *Phoma medicaginis* var. *pinodella* incites symptoms very similar to those caused by *M. pinodes*. However, *P. medicaginis* var. *pinodella* is associated with more severe foot rot symptoms that may extend below the soil line, but appears to cause less damage to leaves, stems and pods [4]. Symptoms of *Ascochyta pisi* infection includes lightly sunken, circular, tan-colored lesions with a dark brown margin on the leaves, pods, and stems [8]. This fungus usually does not attack the base of pea plants or cause foot rot. *Mycosphaerella pinodes* is the predominant pathogen of pea in Canada [9,10] although *P. medicaginis* var. *pinodella* and *A. pisi* are frequently detected at low levels in the major pea-producing regions of Canada and worldwide [1]. Recently, Liu et al. [11] assessed the genetic structure of a sub-population of the isolates of the pea blight complex included in the present study, and evaluated aggressiveness of the isolates on a single susceptible cultivar. But the authors did not investigate the variability in the virulence of the isolates.

The deployment of resistant cultivar is the most effective and ecologically sustainable disease management strategy. Effective sources of resistance to *A. pisi* have been identified in conventional pea types and used successfully in the development of new resistant cultivars [3]. Resistance to *M. pinodes* or *P. medicaginis* var. *pinodella* has been observed only at moderate levels in conventional pea types [4]. In Canada, Xue and Warkentin [12] evaluated 335 pea lines originating from 30 countries against *M. pinodes* and identified seven lines with partial resistance. Resistance to *M. pinodes* is determined by a series of single dominant genes [13], and a single dominant gene controls resistance to *A. pisi* [14].

There have been reports that variation in virulence is present in populations of *M. pinodes*, based on the reactions of host differential genotypes. Several *M. pinodes* pathotypes have been reported in different countries including Canada [9,15,16]. Based on the reactions of differential host genotypes to inoculation with *M. pinodes*, 22 pathotypes of the fungus have been identified in Canada [9], six in West Germany [17], and 15 in Australia [18]. Variation in the virulence of *M. pinodes* populations obtained from commercial field pea crops in Alberta was assessed about a decade ago, and the isolates of *M. pinodes* were classified into different pathotypes based on their virulence pattern on a set of 10 differential hosts [15]. Given there is pathogenic variability in populations of *M. pinodes* [9,15,16], and resistance is controlled (in many cases) by one or a few genes [13], it is possible that selection of virulent isolates has occurred over time.

The objectives of this study were to identify the fungi associated with the ascochyta blight complex on pea, examine pathogenic variability, and determine whether the aggressiveness of *M. pinodes* populations from central Alberta has increased over time. This information is essential to understanding the genetic structure of the pathogen population in the region, and will provide useful information for breeding programs, epidemiological studies, and improved disease management.

2. Materials and methods

2.1. Pathogen isolation

Field pea plants with typical ascochyta blight symptoms were collected from commercial crops in eight counties across central Alberta from 2011 to 2012. Diseased leaf or stem pieces were surface-sterilized in 0.8% NaOCl for 30 to 60 s, rinsed 3 times in sterile water, and air dried. Each piece was then placed on a 1.2% water agar medium (4 pieces/dish) amended with 50 μmol L^{-1} streptomycin sulfate and incubated on a laboratory bench at room temperature (20 ± 2 °C) under a 16 h light and 8 h dark photoperiod for 1 to 2 weeks. Isolates thought to be associated with the ascochyta blight complex were first identified based on the morphological characteristics of the colonies, and were transferred onto potato dextrose agar (PDA) medium for purification. Single pycnidiospore-derived isolates were stored as spore masses in water or 20% glycerol at –20 °C or as mycelial colonies on PDA slants at 4 °C.

2.2. Morphological characterization

A total of 275 single-spore fungal isolates were grown on pea agar medium (2% pea powder, 1.5% agar, w/w) for 10–15 d with a 16-h photoperiod under fluorescent light at 20 ± 2 °C. Colony characteristics (color, mycelial growth, orientation and abundance of pycnidia) were assessed visually or with a stereo microscope, and the shape and size of conidia were determined with a compound microscope. Since the production of carrot-red spore masses on oatmeal agar [19] is the principal characteristic used to distinguish *A. pisi* from *M. pinodes* or *P. pinodella*, the single-spore isolates were plated onto oatmeal agar and incubated for 12 d under the same day/night cycle and temperature regime described above. The color of the spore masses was observed with a stereo microscope.

2.3. Sequence analysis of RPB2 gene

Total genomic DNA of 21 randomly selected isolates of *M. pinodes* including GI (15 isolates) and GII (6 isolates), and two isolates of *P. medicaginis* var. *pinodella* was extracted from mycelia following the method of Feng et al. [20]. DNA concentration and quality were estimated with a Thermo Scientific NANODROP 1000 Spectrophotometer (Fisher Scientific, Nepean, ON, Canada). The *RPB2* gene was amplified using the primer pair RPB2 R2-4Fa and RPB2-7R [8] in a 30 μL reaction volume containing Econo*Taq* Plus 2× Master Mix (Lucigen, Middleton, WI, USA), 0.5 μmol L^{-1} of each primer, and 10 ng of genomic DNA. The DNA amplification conditions in the thermocycler were set as follows: 5 min at 95 °C followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C, and 2 min at 72 °C with a final extension of 10 min at 72 °C. The amplicons were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) using the Eco RV-digested and T-tailed pGEM-T Easy Vector System I (Promega), and transformed into the competent *Escherichia coli* strain JM109 (Promega). Plasmid DNA containing the insert was obtained with a PureYield Plasmid Miniprep System (Promega) and sequenced at the University of Alberta, Edmonton, AB, Canada. The resulting DNA sequences were analyzed and edited with BioEdit (version 7.1.3) [21]. The 23 experimentally obtained sequences were aligned using BioEdit. The best-fit nucleotide substitution model was then selected using the program jModelTest [22]. Distance trees were produced with PAUP 4.0b10 software using the neighbor-joining approach [23]. Support for groups in the tree was assessed using a bootstrap analysis with 1000 replicates.

2.4. Pathotype characterization

Nine cultivars or lines of field pea previously used as differential genotypes to evaluate pathogenic variability in populations of *M. pinodes* from Alberta [16] were used in this study. The differential genotypes consisted of JI 181 and JI 190 (susceptible), Carrera, Danto and Eclipse (moderately susceptible), and Majoret, Miko, Radley and JI 96 (resistant). Of these nine differentials, Xue et al. [9] included JI 96, JI 181, JI 190, Danto, Majoret and Miko, while Zhang et al. [15] included Danto, Majoret, Miko and Radley to characterize the population structure of *M. pinodes* in Canada. Seeds of the differential genotypes were surface-sterilized with 1% NaOCl for 10 min, washed four times with sterile water, and air dried. Four seeds of each cultivar/line were planted in a four-cell row of a 4 × 10 cell "root trainer" tray (Kuhlmann Greenhouses, Edmonton, AB), with one seed per cell. Each cell was filled with a potting mixture consisting of vermiculite, peat moss, loam, and sand (1.00:1.25:1.00:0.75, v/v). The cells were watered daily and the seedlings were maintained in a greenhouse for 12 days until inoculation.

A total of 110 isolates of *M. pinodes* were assessed for variation in virulence on the differential set including 20 isolates from Sturgeon county, 10 from Minburn county, 9 each from Parkland and Strathcona counties, 8 from Westlock county, 7 from Smoky Lake county, 6 from Big Lakes county and 9 from the Edmonton area, all of which were collected in 2011. An additional 32 isolates from Minburn county, collected in 2012, also were used in the analysis. The isolates were grown on pea agar medium for 10 d under fluorescent lighting and an ambient temperature of 20 ± 2 °C. Each dish was flooded with 3 mL of sterile water plus 1% Tween-80 and cultures were disrupted with a sterile glass rod to release the conidia. The conidial suspension was filtered through eight layers of cheese cloth, and the inoculum measured with a hemocytometer and adjusted to 10^6 conidia mL^{-1} with sterile distilled water.

For each isolate, four replicate plants of each differential genotype were point-inoculated with a 2-μL drop of the spore suspension applied to each of six leaflets (each on a different node) at 12 days after seeding. After inoculation, the plants were left in darkness for 12 h until the water had evaporated from the inoculum droplets. The inoculated plants were then sprayed with deionized water containing 0.5% Tween-80 with a hand sprayer and incubated inside dark plastic bags for 48 h at 18 °C. The plants were removed from the bags and transferred to a greenhouse at 20 ± 2 °C with a 16-h photoperiod and light intensity of 140 μmol m^{-2} s^{-1}. For each isolate, the four largest lesions were chosen from each replicate plant and the diameter of each lesion was measured with a digital caliper at 8 days after inoculation. A resistant (R) reaction was defined as one in which the mean lesion diameter size ranged from 0 to 2 mm, while a mean lesion diameter greater than 2 mm was regarded as a susceptible (S) reaction. Fungal isolates were grouped into different pathotypes based on the reaction patterns of the nine host differentials. The experiment was repeated once.

2.5. Data analysis

Prior to analysis of the pathotype characterization data, the homogeneity of variance of each data set was confirmed using normal probability plots. All of the analyses were performed with SAS software version 9.2 (SAS Inc., Cary, NC, USA). The data were examined using a general linear model analysis of variance (PROC GLM in SAS). There was no main effect of repetition, so the data from the two repetitions of each experiment were combined for subsequent analyses. Data were presented in box-and-whisker plots to depict the location effect of isolates on disease severity on the nine pea differentials. The skewness of the data for each differential genotype was calculated (PROC UNIVAR) to assess the degree of asymmetry around each mean.

3. Results

3.1. Cultural characterization

Of the 275 fungal isolates collected from pea plants in central Alberta, 272 were identified as *M. pinodes* and three as *P. medicaginis* var. *pinodella* (Fig. 1). None of the isolates in the collection produced a carrot-red spore mass consistent with classification as *A. pisi*. The isolates of *M. pinodes* were classified into two groups, GI and GII, based on colony color and pycnidial distribution. GI isolates produced light to dark, mostly gray colonies with a radial or concentric pycnidial

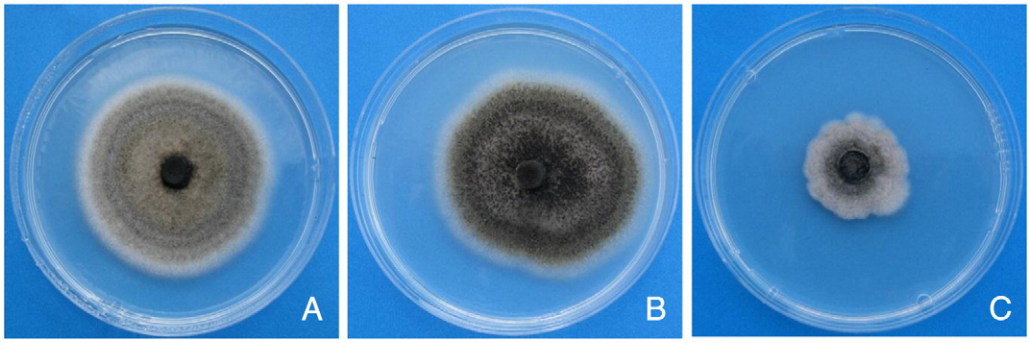

Fig. 1 – Morphological characteristics of the pathogens of pea blight complex. A. *Mycosphaerella pinodes* (GI), B. *M. pinodes* (GII) and C. *Phoma medicaginis* var. *pinodella* on potato dextrose agar (PDA) medium.

distribution. GII isolates had dark to gray colonies with abundant pycnidia scattered throughout the colony (Fig. 1). The size and morphology of the conidia overlapped between the two groups. The size of the conidia of GI isolate was 10.5–14.5 × 4.2–6.2 μm mostly with one septum, occasionally two with a constriction at the septum. The spore mass was generally light buff to flesh color. The conidia size of the GII isolate was 8–16 × 3.5–6.2 μm mostly with one septum with a constriction at the septum. The color of the spore mass was pale creamy white. GI isolates were consistently much more abundant (83%) than GII isolates (17%). The highest percentage of GII isolates was found in Smoky Lake county (30%), followed by Westlock (26%), Mannville (23%), Parkland and Strathcona (15%), Minburn (3%) and Sturgeon counties (2%). No GII isolates were obtained from Big Lakes county (Fig. 2).

The three isolates of *P. medicaginis* var. *pinodella* produced brown to gray colonies and pycnidia in a concentric ring with off-white hyphae. The conidia ranged in size from 4.5 to 10.0 μm × 2.5–4.5 μm.

3.2. Sequence analysis of RPB2 gene

The primers RPB2 R2-4Fa and RPB2-7R amplified a ca. 900 bp amplicon from all the 23 isolates. The nucleotide frequencies were A, 0.2425; C, 0.2625; G, 0.2797 and T, 0.2153. Two isolates of *P. medicaginis* var. *pinodella* were rooted as an outgroup, and the other 21 isolates of *M. pinodes* were grouped together in the neighbor-joining tree with bootstrap value shown in the tree (Fig. 3). Sequence analysis of the *RPB2* gene of the representative isolates revealed that both GI and GII isolates belonged to *M. pinodes*.

3.3. Pathotype characterization

Of the 110 isolates assessed, 83% were virulent (mean lesion diameter > 2.0 mm) on all nine differential lines (Table 1). In an analysis of variance, the main effect of differential genotype explained 79% of the variance in lesion diameter, the main effect of isolate explained 19%, and isolate × genotype interaction ($P < 0.01$) explained 2% of the variance. The median lesion

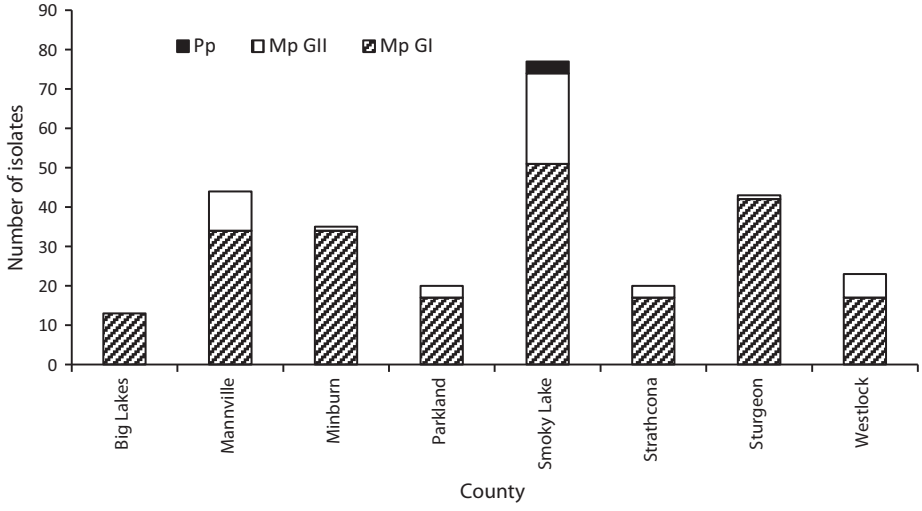

Fig. 2 – Geographic distribution of fungal isolates associated with the ascochyta blight complex of a field pea in central Alberta in 2011–2012. Total number of isolates: 275. Pp: *Phoma medicaginis* var. pinodella (1%); Mp GI: *Mycosphaerella pinodes* group I (82%); Pp GII: *M. pinodes* group II (17%).

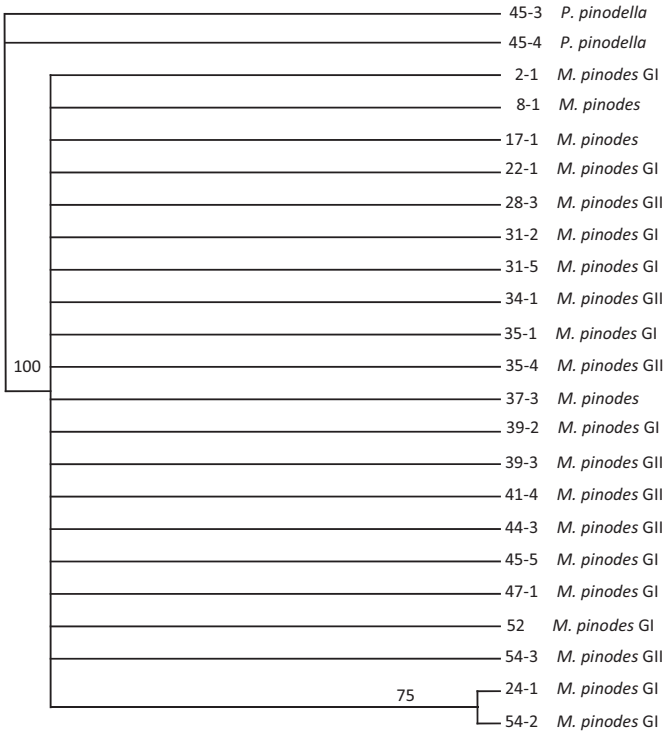

Fig. 3 – Phylogenetic tree of *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella* isolates based on RPB2 sequence data using the neighbor-joining method. Bootstrap values based on 1000 replicates are displayed on the branches.

diameter averaged over nine pea differentials was greater for Big Lakes, Edmonton, Minburn, and Smoky Lake isolates compared to Parkland, Strathcona, Sturgeon and Westlock isolates (Fig. 4). Over all locations, the lesion diameter ranged from 1.6 to 5.6 mm. Isolates with greater aggressiveness were found at all locations included in the study. Only two of the isolates, one each from Minburn and Smoky Lake, produced lesion diameters smaller than 2.0 mm. Lesions were larger on lines JI 181, JI 96 and Miko than on the other differential genotypes, and smaller on Danto than on all others except Carrera and Majoret (Fig. 4).

The isolates were grouped based on the lesion sizes they incited on the differentials and the frequency of isolates in each group on every differential genotype. When an isolate

Table 1 – Pathotype grouping of *Mycosphaerella pinodes* isolates based on the reactions of nine differential cultivars/lines of field pea.

Pathotype	No. of isolates	Location (county)	Differential cultivars/lines								
			JI 181	JI 96	JI 190	Carrera	Eclipse	Majoret	Radley	Miko	Danto
I	91	All locations	S	S	S	S	S	S	S	S	S
II	3	Minburn	S	S	S	S	S	S	S	S	R
	2	Sturgeon									
	1	Strathcona									
III	1	Westlock	S	S	S	S	S	S	S	R	S
IV	1	Strathcona	S	S	S	S	S	R	S	S	S
V	1	Westlock	S	S	S	S	R	S	S	S	S
VI	2	Minburn	S	S	S	R	S	S	S	S	S
VII	1	Strathcona	S	S	R	S	S	S	S	S	S
VIII	1	Sturgeon	S	S	S	S	R	R	S	S	S
IX	1	Sturgeon	S	R	S	S	S	S	S	R	S
X	1	Minburn	S	R	S	S	R	S	S	S	R
XI	2	Minburn	S	R	S	R	S	R	R	S	R
XII	1	Minburn	S	R	S	S	S	R	R	R	R
XIII	1	Smoky Lake	R	R	R	R	R	R	S	S	R

Each isolate and differential cultivar/line was categorized as resistant (R) when the mean lesion diameter was 0–2.0 mm and susceptible when the diameter was ≥2.0 mm.

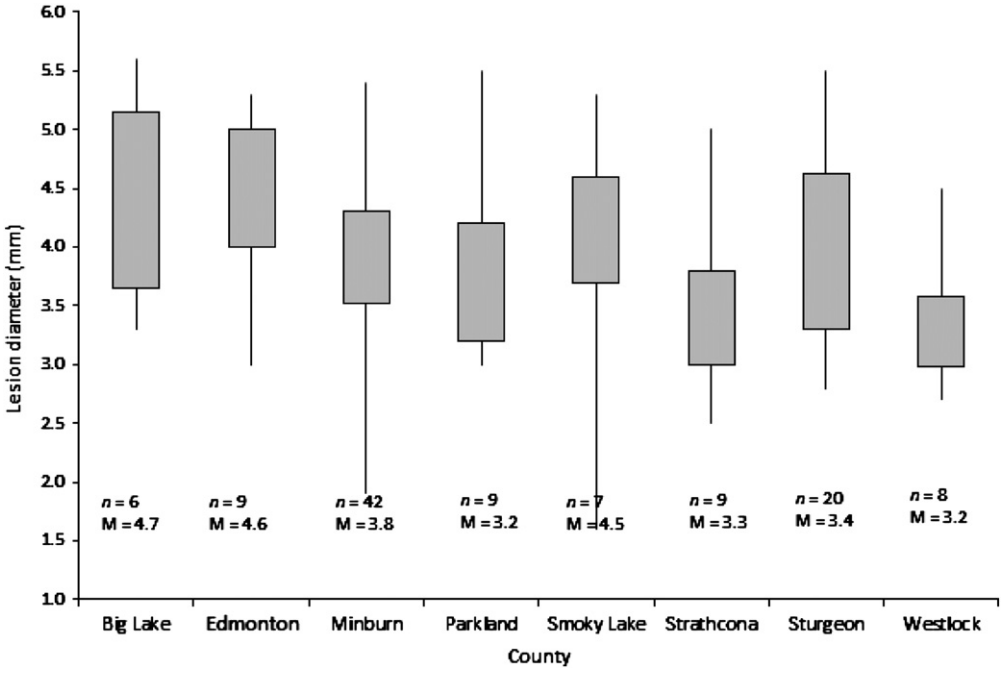

Fig. 4 – Distribution of lesion diameter caused by 110 isolates of *Mycosphaerella pinodes* on nine pea differential cultivars/lines. Data are means of 2 trials × 9 differential cultivars/lines × 4 replicate plants (each replicate consists of lesion diameter measurements on four inoculated foliates at different nodes of each plant). Data are shown according to geographical origin of the isolates; *n* = number of isolates and M = median lesion diameter.

caused lesions less than 2.0 mm in size on a host differential, that genotype was classified as resistant to that isolate. The cumulative frequency of isolates causing lesions less than 2.0 mm in diameter was considered as an estimate of the probability of resistance of a differential genotype. Using this estimate, the probability of resistance to isolates of *M. pinodes* in the sample from Alberta was 7% for Danto, 5% for JI 96 and Majoret, 3% for Eclipse and Miko, 2% for JI 190, Carrera and Radley, and 1% for line JI 181 (Fig. 5).

Mean lesion size on six of the nine differential lines approximated a normal distribution; only Carrera, Danto, and Miko showed a skewed distribution of lesion size (skewness ≥ 0.4) (Fig. 5). The peak of the lesion size distribution for the differentials ranged from 3.35 to 4.79 mm. Also, the frequency of isolates with lesion size three times larger than an 'avirulent' lesion type was 0 to 1.0% on six of the nine differentials, but was 12% on Miko, 16% on JI 181 and 35% on JI 96 (Fig. 5).

Based on the virulence of the 110 isolates of *M. pinodes* on the nine differentials, the isolates were grouped into 13 individual pathotypes (Table 1). Pathotype I, which included 83% of the isolates, was virulent on all of the differential lines and was collected from all of the sampling sites. All of the other pathotypes were represented by only 1–6 isolates. Pathotypes II to VII were each avirulent on only one of the differential genotypes. Pathotypes VIII to IX were avirulent on two, pathotype X on three, pathotypes XI and XII on five and pathotype XIII on seven genotypes (Table 1).

The population of *M. pinodes* collected in Minburn county was the most diverse, and included the isolates classified as pathotypes I, II, VI, X, XI and XII. The isolates representing pathotypes I, II, VIII and IX were collected in Sturgeon county, and the isolates classified as pathotypes I, II, IV and VII were collected in Strathcona. Pathotypes I, III and V were found in the collections from Westlock County, while only pathotypes I and XIII were identified in Smoky Lakes. The isolates collected from Edmonton, Parkland and Big Lakes Counties were all classified as pathotype I. The virulence of the pathotypes did not correspond to the GI and GII designations for colony characteristics.

4. Discussion

4.1. Cultural characterization

In this study, 275 isolates were identified to species based on their cultural and morphological characteristics. Of these, 272 isolates (99%) were *M. pinodes* and the remaining three isolates were *P. medicaginis* var. *pinodella*. No isolate of *A. pisi* was found. Two distinct colony phenotypes were observed among isolates of *M. pinodes*, designated GI and GII. The colony characteristics of the GII isolates resembled those of the *A. pisi* isolates described by Davidson et al. [1], but they did not produce carrot-red spore masses, the principal morphological characteristic used to distinguish *A. pisi* from the other fungi in this ascochyta blight disease complex. Moreover, sequence analysis of *RPB2* gene of the representative isolates revealed that both GI and GII isolates belonged to *M. pinodes*.

The present study demonstrated that *M. pinodes* is the predominant fungus isolated from lesions on field pea leaves in central Alberta. No isolates of *A. pisi* or *P. koolunga* were

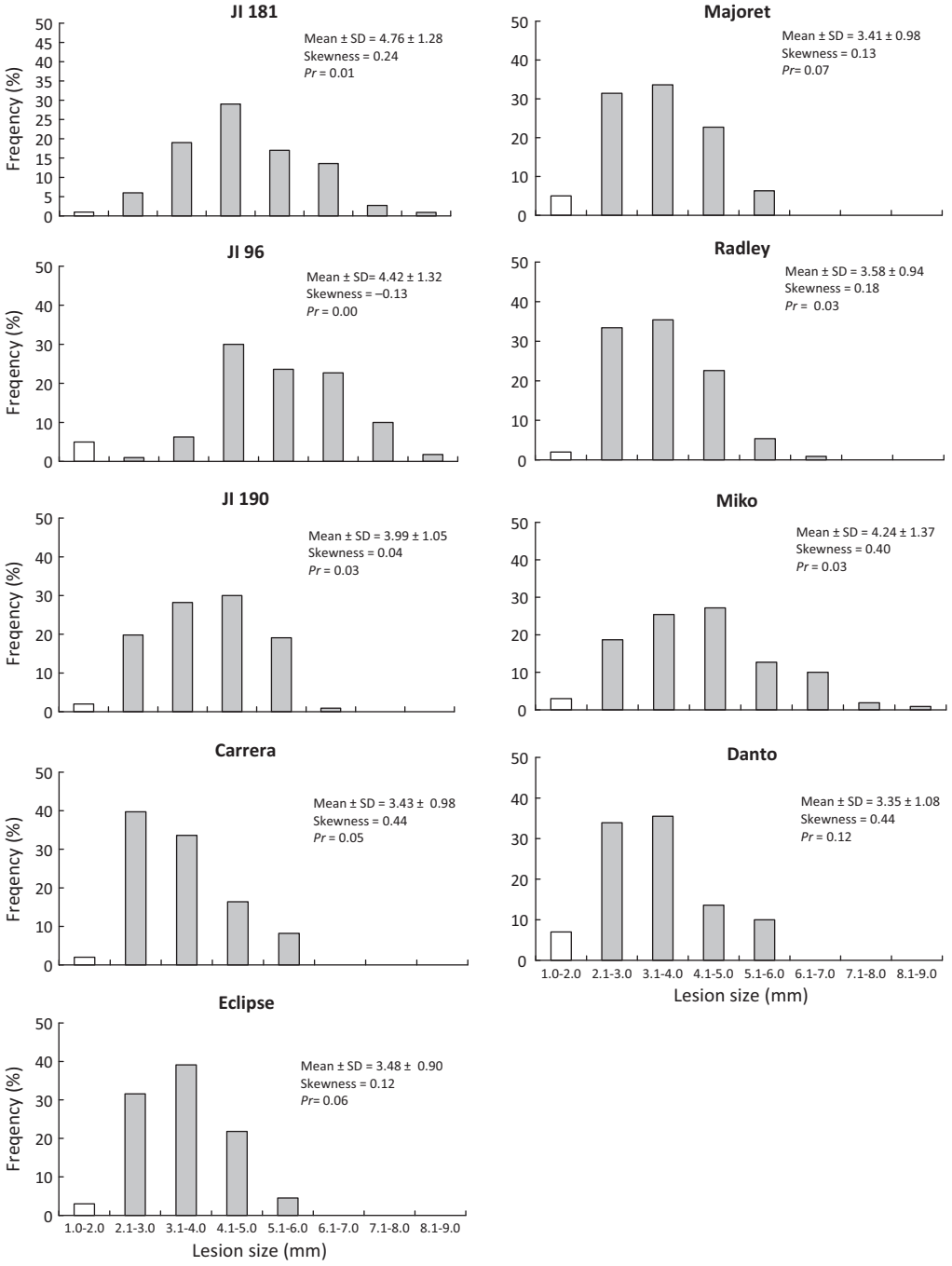

Fig. 5 – Distribution of lesion size caused by *Mycosphaerella pinodes* isolates from Alberta on nine differential cultivars/lines of field pea. The open bars represent the frequency of avirulent isolates (lesion diameter ≤ 2.0 mm). For each differential line, the mean lesion size (mm) ± standard deviation, a skewness statistic for the distribution, and the frequency of avirulent isolates (Pr: probability of resistance) is also presented.

detected and the proportion of *P. medicaginis* var. *pinodella* isolates was negligible. A previous report also found that *M. pinodes* was the dominant pathogen in the ascochyta blight complex on the Canadian prairies [24]. Nonetheless, *A. pisi* also has been reported from the Canadian prairies, most commonly from infected seeds of specific pea cultivars produced in parts of southern Saskatchewan [25,26]. In South Australia, *M. pinodes* was the predominant pathogen in the disease complex, but *P. medicaginis* var. *pinodella* was also common. *P. koolunga* also was shown to be an important component of the blight complex, while *A. pisi* was only rarely detected [2–4].

In the present study, samples of leaves (or occasionally pods) with symptoms were collected from central Alberta, but no isolates were collected from seed. It is likely that in central Alberta, the popular field pea cultivars are more susceptible to

M. pinodes than to the other pathogens in the disease complex. It is also possible that seed is more susceptible to infection by *A. pisi* than by the other fungi.

Molecular techniques have served as effective tools for fungal diagnosis and taxonomy [27,28]. For example, *A. pisi* can be differentiated from *M. pinodes* and *P. medicaginis* var. *pinodella* by restriction fragment length polymorphism (RFLP) analysis of ribosomal DNA spacers [29] and random amplified polymorphic DNA (RAPD) assays [30]. Internal transcribed spacer (ITS) sequences can be used to discriminate *M. pinodes* and *P. medicaginis* var. *pinodella* from *A. pisi* and *P. koolunga* [2,31–33]. Recently, ITS sequence analysis was used to demonstrate that *A. pisi* and *P. koolunga* are likely not present in central Alberta [11].

4.2. Pathotype characterization

All or some of the differential pea lines/cultivars included in this study have been used in previous studies of *M. pinodes* populations [7,15,16], enabling comparison of pathogen aggressiveness and pathotype structure over time. In the present study, each of the 110 isolates evaluated was virulent on at least one of the nine differential pea lines. The majority of isolates (80%) were virulent on all nine differentials. However, there was a significant differential × isolate interaction for lesion size in the analysis of variance. This finding indicates the presence of different pathotypes in the population of *M. pinodes* in central Alberta. In a similar assessment in a previous study, the aggressiveness of the isolates was the dominant factor contributing to the variance [15]. By contrast, in the present study, the reaction of the differential lines was the dominant factor contributing to the variance. This difference likely reflects the fact that Zhang et al. [15] evaluated isolates collected over a longer period of time (1991–2000) from a wider geographical area, including the Canadian prairies, New Zealand, Ireland, France and Australia. The isolates analyzed in the present study originated only from central Alberta and were collected in 2011–2012.

In the present study, Danto, Miko, Majoret, JI 190, JI 181 and JI 96 were resistant to 7%, 3%, 5%, 2%, 1% and 5% of the isolates tested, respectively. Six of the nine differential lines included in the present study were used 15 years ago to assess the reaction of 275 isolates of *M. pinodes* from Manitoba (147 isolates), Saskatchewan (76 isolates) and Alberta (52 isolates). At that time, Danto, Miko, Majoret, JI 96, JI 181 and JI 190 were resistant to 10%, 3%, 3%, 2%, 2% and 2% of the isolates, respectively [9]. Considering only the isolates from Alberta, the corresponding values were 11%, 4%, 8%, 0, 0 and 2%. These results indicate that there has been little change in the *M. pinodes* population over the last 15 years. The isolates were grouped on the basis of the lesion sizes induced on the differential lines and on the frequency of isolates in each category on every differential line, following the method of Su et al. [16]. The present results revealed that the probability of resistance reaction to isolates of *M. pinodes* from central Alberta was 1% for JI 181, 2% for JI 190, Carrera and Radley, 3% for Miko and Eclipse, 5% for JI 96 and Majoret, 7% for Danto and 2% for Radley (Fig. 3). Su et al. [16] evaluated the virulence of 83 isolates on the same differential lines and determined that the probability of resistance to isolates of *M. pinodes* was 59% for JI 96, 9% for JI 181, 23% for JI 190, 55% for Miko, 40% for Carrera, 37% for Eclipse, 51% for Majoret, 37% for Danto and 86% for Radley. Also, the mean lesion size was 1.4 mm on JI 96, 2.9 mm on JI 181, 2.2 mm on JI 190, 1.5 mm on Miko, 1.8 mm on Carrera, 1.9 mm on Eclipse, 1.6 mm on Majoret, 1.8 mm on Danto and 0.8 mm on Radley. The corresponding lesion sizes in the present study were 4.4, 4.8, 4.0, 4.2, 3.4, 3.5, 3.4, 3.4 and 3.6 mm, respectively. These results indicate that the aggressiveness of *M. pinodes* isolates has increased on each differential host over the past 7 years.

Approximately 80% of the 110 isolates of *M. pinodes* collected from central Alberta were virulent on all of the differential lines, and so were placed in pathotype I. This result supports a previous report that 80% of the 275 western Canadian *M. pinodes* isolates assessed belong to pathotype I based on their reaction on nine pea differential lines [9], six of which also were used in the present study. In another study, the reactions of 58 *M. pinodes* single-pycnidiospore isolates collected from western Canada, New Zealand, France, Australia, the United Kingdom, and Ireland were assessed for virulence using six differential genotypes (4 of which were used in our study), and it was found that 72% of the Canadian isolates were classified as pathotype I [15]. These data indicate that pathotype I was predominant in central Alberta in 2011–2012, and has been prevalent in western Canada for at least the past 15 years.

5. Conclusions

The majority of the isolates (272 of 275, 99%) of the ascochyta blight disease complex in central Alberta were of *M. pinodes* and the remaining three isolates (1%) were of *P. pinodella*. None of the isolates was of *A. pisi* or *P. koolunga*. The dominant pathotype in the populations (pathotype I) has been prevalent for many years, and is virulent on all of the differential lines assessed. Its aggressiveness on these differentials also may be increasing over time. Since *M. pinodes* is the predominant pathogen in the ascochyta blight disease complex, it should be a primary focus of resistance breeding efforts in central Alberta.

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