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Characterization of the fungi associated with ascochyta blight of field pea in Alberta, Canada

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ABSTRACT

Ascochyta blight, caused by a complex of *Mycosphaerella pinodes, Phoma pinodella*, *Ascochyta pisi*, and/or *Phoma koolunga*, is a devastating disease of field pea. In order to understand the composition of fungi associated with ascochyta blight in Alberta, Canada, a total of 157 single-pycnidiospore fungal isolates were obtained from diseased pea samples collected from central and northern Alberta in 2011. These isolates were characterized for species identity, aggressiveness, DNA sequence variation in the internal transcribed spacer (ITS) region, and random amplification of polymorphic DNA (RAPD) patterns. The ITS sequences obtained from 142 fungal isolates were all identical to the ITS sequences from *M. pinodes* and/or *P. pinodella*. Inoculation of the 157 isolates on a susceptible pea cultivar Midas indicated that most of the isolates were moderately to highly aggressive. Phylogenetic analysis based on the RAPD data revealed two main groups and six sub-groups, with one main group comprising 78% of the 157 isolates. Distinct RAPD patterns were associated with isolates from particular geographic locations, but were not generally associated with isolate aggressiveness.

1. Introduction

Ascochyta blight (blackspot) is one of the most devastating diseases of field pea worldwide, and has been reported in many major pea-growing areas (Bretag et al., 2006). Yield losses due to ascochyta blight have been estimated to be at least 10% each year in many pea crops in Australia (Bretag et al., 2006), and 40% in experimental field plots in France (Tivoli et al., 1996). In Canada, yield losses caused by ascochyta blight have been estimated at 10% in commercial crops, but losses of over 50% have been reported in field trials (Wallen, 1965, 1974; Xue et al., 1995). While yield losses caused by ascochyta blight have not been measured in Alberta, Canada, a recent field survey revealed a high incidence and severity of the disease in this province (Cao et al., 2012). Considering that field pea is an economically important crop and generates more than $600 million dollars annually in Canada (FAO, 2010), and that pea production in Alberta represents >20% of the national total (Statistics Canada, http://www.statcan.gc.ca/pub/22-002-x/2012006/t053-eng.pdf), the economic damage caused by ascochyta blight is significant and requires more attention.

Ascochyta blight is caused by a complex of fungal pathogens, including *Ascochyta pisi* Lib. (teleomorph: *Didymella pisi* sp. *nov.*), which causes leaf, stem and pod spot, *Ascochyta pinodes* L.K. Jones (teleomorph: *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.), which causes leaf, stem and pod spot, and foot rot, *Phoma pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch, which causes leaf spot, stem lesions and foot rot, and *Phoma koolunga* Davidson, Hartley, Priest, Krysinska-Kaczmarek, Herdina, McKay & Scott sp. nov. (teleomorph unidentified), which causes the formation of leaf and stem chlorotic and necrotic spots (Davidson et al., 2009). *A. pisi* is a heterothallic fungus which causes slightly sunken, circular, tan-coloured lesions with dark brown margins that occur on the leaves, pods, and stems (Chilvers et al., 2009). *A. pisi* seldom attacks the base of the plant and does not cause foot rot; the pathogen survives poorly in soil, but can sometimes survive on volunteer plants or pea stubble (Bretag et al., 2006). *A. pinodes* is a homothallic fungus that produces both conidia and ascospores (*M. pinodes*) on field pea during the growing season and can survive in soil as a saprophyte for several years (Wallen et al., 1967; Chilvers et al., 2009). *A. pinodes* attacks the leaves, stems, flowers, pods, seeds, and seedlings of pea, causing the development of necrotic leaf spots, stem lesions,

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shrinkage and dark-brown discolouration of seeds, blackening of the base of the stem, and foot rot of seedlings. *P. pinodella* is a heterothallic fungus (Chilvers et al., 2009), and while its teleomorph has been reported in culture (Bowen et al., 1997), the sexual stage has not been named. *P. pinodella* causes diseases on pea with symptoms very similar to those caused by *A. pinodes*. However, *P. pinodella* survives well in the soil and tends to cause less damage to the leaves, stems and pods, while causing more severe foot rot symptoms that can extend below ground (Bretag et al., 2006). Recently, another fungus, *P. koolunga*, has been shown to contribute to the ascochyta blight complex, at least in Australia (Davidson et al., 2009). The disease symptoms caused by *P. koolunga* are indistinguishable from those caused by *M. pinodes*, other than a 24 h delay in their development (Davidson et al., 2009). *P. koolunga* may also survive well in soil for a number of years (Davidson et al., 2012). *A. pisi*, *A. pinodes*, *P. pinodella*, and *P. koolunga* are all seed-borne pathogens and all can survive on infected pea debris (Ali et al., 1982; Bretag et al., 2006; Davidson et al., 2009). Among the four species, *A. pinodes* is responsible for most of the damage (Xue et al., 1997), although *A. pisi* and *P. pinodella* are frequently reported in the major pea producing areas worldwide. *P. koolunga* has been frequently observed in the pea producing regions of Australia (Davidson et al., 2012).

Control of ascochyta blight is based largely on fungicidal seed and foliar treatments and cultural practices such as crop rotation (Bretag et al., 2006; Zhang et al., 2006; Fondevilla et al., 2008). A number of fungicides, including mancozeb, chlorothalonil, benomyl, carbendazim, and thiabendazole, have been used to effectively control ascochyta blight (Warkentin et al., 1996, 2000), because these fungicides have both preventative and eradicative properties against the fungi associated with ascochyta blight, coupled with good residual activity (Bretag et al., 2006). Fungicide applications, however, may increase production costs and enhance the risk of a build-up in fungicide tolerance in pathogen populations. The development of genetically resistant cultivars would be an economically desirable strategy for management of ascochyta blight.

Sources of resistance to the ascochyta blight fungi are very limited. Good sources of resistance to *A. pisi* have been reported in conventional pea types and used successfully in the development of new resistant varieties (Lawyer, 1984). However, only moderate levels of resistance to either *M. pinodes* or *P. pinodella* have been observed in conventional pea types (Bretag et al., 2006). Xue and Warkentin (2001) screened 335 pea lines from 30 countries and identified 7 lines with partial resistance to *M. pinodes*. Genetic analysis of stem and foliar resistance showed that resistance to *M. pinodes* is determined by a series of single dominant genes (Clulow et al., 1991), and Csizmadia (1995) suggested that a single dominant gene controls resistance to *A. pisi*. While ascochyta blight resistance in field peas is generally thought to be non-race-specific, it appears that resistance to each of the pathogens is under separate genetic control (Bretag et al., 2006).

A better understanding of genetic variation within a pathogen species is important because this information can affect the strategies that are selected for the development of control measures (McDonald and Linde, 2002). For example, pathogen populations with a high level of genetic variation are more likely to overcome genetic resistance than pathogen populations with a low level of genetic variation. Furthermore, a close genetic relationship between pathogenic and non-pathogenic isolates within a species may indicate the recent evolution of pathogenic isolates from non-pathogenic relatives, or vice versa (Woudt et al., 1995). On the other hand, the presence of genetically heterogeneous isolates at a site may indicate the occurrence of sexual reproduction or that some of the isolates may have been recently introduced, for instance via long-distance dispersal of spores on infested seed or soil (Schmale et al., 2006). Information on the genetic variation of the ascochyta blight complex in Alberta, Canada, is still very limited. Earlier reports indicated that there were 22 pathotypes of *M. pinodes* in Canada (Xue et al., 1998), six in West Germany (Nasir and Hoppe, 1991), six in Poland (Furgal-Wegrzycka, 1984), and 15 in Australia (Ali et al., 1978). In central Alberta, Su et al. (2006) identified six pathotypes of *M. pinodes* based on their virulence pattern on a set of 10 differential hosts. However, the composition of the pathogen species causing ascochyta blight on field pea has not been determined in Alberta.

A number of molecular techniques are available for assessment of genetic variation in pathogen populations. Random amplification of polymorphic DNA (RAPD) and sequencing of the internal transcribed spacer (ITS) region have been used in the studies of the ascochyta blight complex. Some RAPD markers have been developed to distinguish *A. pisi* from *M. pinodes* and *P. pinodella* (Bouznad et al., 1995), and to distinguish *M. pinodes* from *P. pinodella* (Onfroy et al., 1999). Comparison of the ITS sequences among the components of the ascochyta blight complex led to the discovery of the new species *P. koolunga* in Australia (Davidson et al., 2009).

In the current study, the phylogeny and aggressiveness of ascochyta blight fungal isolates from central Alberta were assessed. The objectives of the study were to: i) characterize the genetic structure of ascochyta blight pathogen populations; ii) evaluate the utility of RAPD analysis and ITS sequencing in studies of genetic variation within the complex; and iii) determine the relationship between the phylogenetic profile of the single-spore isolates and their aggressiveness and geographic origins.

2. Materials and methods

2.1. Fungal isolates

Diseased pea plants with ascochyta blight symptoms were collected from 56 fields in the municipalities/counties of Strathcona, Westlock, Parkland, Smoky River, High Prairie, Sturgeon, Minburn, and Big Lakes in Alberta, Canada in 2011 (Cao et al., 2012) (Fig. 1). Plant tissues including leaves, stipules, or pods (5 mm × 5 mm) with typical ascochyta blight lesions were surface-sterilized in 1% a.i. sodium hypochlorite for 1 min, rinsed three times with sterile deionized water (sdH_2O), and placed on 1% (w/v) water agar containing 0.01% (w/v) streptomycin sulphate in 10-cm-diameter Petri dishes. The dishes were incubated at 25 °C under 16 h light and 8 h dark for a period of 7–10 days for fungal colony growth and sporulation. The resulting pycnidiospores were identified to the species level with a stereomicroscope, transferred to fresh potato dextrose agar (PDA) plates with a needle, and incubated for 7–10 days at 25 °C under 16 h light and 8 h dark to induce a second round of sporulation. The resultant pycnidiospores were then transferred with a needle to fresh water agar plates supplemented with 0.01% (w/v) streptomycin sulphate, and about 2 mL sdH_2O were added and spread evenly over each plate with a sterile glass rod. The plates were then incubated overnight at room temperature to allow pycnidiospore germination. Single germinated pycnidiospores were identified by examination with a stereomicroscope and transferred with a needle to fresh PDA plates supplemented with 0.01% (w/v) streptomycin sulphate and incubated as pure, single-spore isolates.

2.2. Aggressiveness test

'SW Midas', a yellow-seeded field pea cultivar susceptible to ascochyta blight, was sown in 200-mL plastic cups filled with Pro-Mix potting mixture (Premier Horticulture, Rivière-du-Loup, QC,