HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

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

Hafiz Ahmed[a], *Kan-Fa Chang*[a], *Sheau-Fang Hwang*[a,*], *Heting Fu*[a], *Qixing Zhou*[a], *Stephen Strelkov*[b], *Robert Conner*[c], *Bruce Gossen*[d]

[a]*Crop Diversification Centre North, Alberta Agriculture and Rural Development, Edmonton, AB T5Y 6H3, Canada*
[b]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada*
[c]*Agriculture and Agri-Food Canada (AAFC), Morden, MB R6M 1Y5, Canada*
[d]*Agriculture and Agri-Food Canada (AAFC), Saskatoon, SK S7N 0X2, Canada*

ARTICLE INFO

Article history:
Received 12 February 2014
Received in revised form 22 April 2014
Accepted 28 September 2014
Available online 5 October 2014

Keywords:
Ascochyta
Phoma
Resistance
Virulence
Pathotype

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.

* *Corresponding author*. Tel.: +1 780 422 2559; fax: +1 780 422 6096.
E-mail address: Sheau-Fang.Hwang@gov.ab.ca (S.-F. Hwang).
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2014.08.007

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.